COMPARATIVE EVALUATION OF ANTIMICROBIAL EFFICACY OF BITTER GUARD (MOMORDICA CHARANTIA) & GARLIC (ALLIUM SATIVUM) AS ENDODONTIC IRRIGANTS AGAINST E.FAECALIS-AN IN VITRO STUDY

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CERTIFICATE

This is to certify that **Dr. Y. ANUSHA,** Post Graduate student (2015-2018) in the Department of Conservative Dentistry and Endodontics, Adhiparasakthi Dental College and Hospital, Melmaruvathur -603319, has done this dissertation titled **"A comparative evaluation of antimicrobial efficacy of bitter guard (MOMORDICA CHARANTIA) & GARLIC (ALLIUM SATIVUM) As endodontic Irrigants Against E.Faecalis An In Vitro Study** under our direct guidance and supervision in partial fulfilment of the regulations laid down by the Tamilnadu Dr.M.G.R Medical University, Chennai – 600032 for MDS., (Branch-IV) **CONSERVATIVE DENTISTRY AND ENDODONTICS** degree examination.

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DECLARATION

TITLE OF THE DISSERTATION	"Comparative evaluation of antimicrobial efficacy of bitter guard(MOMORDICA CHARANTIA) & GARLIC (ALLIUM SATIVUM) endodontic Irrigants Against E.Faecalis An in vitro study
PLACE OF THE STUDY	Adhiparasakthi Dental College and Hospital, Melmaruvathur – 603319
DURATION OF THE COURSE	3 years
NAME OF THE GUIDE	Dr.S.ThillainayagamMDS.,
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I hereby declare that no part of the dissertation will be utilized for gaining financial assistance or any promotion without obtaining prior permission of the Principal, Adhiparasakthi Dental College and Hospital, Melmaruvathur – 603319. In addition, I declare that no part of this work will be published either in print or in electronic media without the guides who has been actively involved in dissertation. The author has the right to reserve for publish work solely with the permission of the Principal, Adhiparasakthi Dental College and Hospital, Melmaruvathur – 603319

Guide **Head of the department**

Signature of the candidate

ABSTRACT

BACKGROUND :

One of the most important objectives of root canal treatment is the elimination of microorganisms from the root canal system. Persistent endodontic infections are mainly due to retention of microorganism in the dentinal tubules. Enterococcus faecalis is the primary organism detected in persistent asymptomatic infections. The most effective method for eliminating E. faecalis from the root canal space and dentinal tubules is the use of Sodium hypochlorite and 2% Chlorhexidine. Due to the disadvantages of these irrigants like toxicity and synthetic concern, consumption of preparations from medicinal plants has increased over the last few decades.

AIM:

To evaluate the antimicrobial efficacy of two herbal extracts i.e, Bitter Guard (Momordica charantia) and Garlic (Allium sativum) as endodontic irrigants against Enterococcus faecalis.

MATERIALS AND METHOD:

Single rooted human mandibular premolars extracted for orthodontic reasons were selected for the study. Teeth were decoronated to standardize the length to 12-15mm. Cleaning and shaping of root canals were done by crown down technique using protaper universal rotary files till F3. Specimens were placed in steel containers containing BHI broth and sterilized in autoclave. From a

stock culture of MTCC 2527 E. faecalis strain, subculture was made onto a plate of Diagnostic Sensitivity Test Agar. Enumeration of live bacteria (CFU) was carried by serial dilution method. For injecting into the tooth a suspension of bacteria containing 10µ CFU per ml was used. The root canals were inoculated with E. faecalis suspension and incubated at 37° c for 21 days. The specimens were divided into five groups, each containing ten teeth. Test irrigating solutions were used as follows. Group 1 - Normal Saline, Group 2 - 5.25% NaOCl, Group 3 - 2% CHX, Group 4 – Bitter guard, Group 5 – Garlic. Dentinal shavings were collected using no 40 H file in an aseptic condition. Shavings were transferred into test tubes containing 10 ml sterile normal saline(10^{-1}). Three serial dilution was carried out i.e., till 10^{-3}. From this 1 ml was pipetted on to a sterile 100 mm diameter disc & to these plates 15 ml of melted agar medium was added and allowed to solidify. Plates were incubated for 24hours at 37° C. After incubation the number of colonies were counted.

RESULTS:

The mean CFU from low to high with all irrigants tested was as follows Group 2 - 5. 25% Naocl (0.00); Group 3- 2% chlorhexidine (1.14×10^{-3}); Group 4- Bitter Guard (1.40×10^{-3}); Group 5- Garlic (10.30×10^{-3}) and Group 1- Normal saline (28.60×10^{-3}) .

CONCLUSION:

- Extracts of Bitter Guard (Momordica charantia) and Garlic (Allium sativum) are effective against E. faecalis.
- Bitter Guard (Momordica charantia) and 2% Chlorhexidine are equally effective against E. faecalis.
- 5.25% NaOCl showed complete inhibition of E. faecalis and proved as a gold standard endodontic irrigant.

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LIST OF ABBREVIATIONS

E. faecalis	: Enterococcus faecalis
C. albicans	: Candida albicans
S. mutans	: Streptococcus mutans
S. aureus	: Staphylococcus aureus
CHX	: Chlorhexidine
+ve	: positive
-ve	: Negative
NaOCl	: Sodium hypochlorite
%	: Percentage
>	: Greater than
<	: less than
$Ca(OH)_2$	: Calcium hydroxide
K – file	: Kerr file
H – file	: Hedstrom file
M. charantia	: Momordica charantia
A. sativum	: Allium sativum
A. vera	: Aloe vera
A. indica	: Azadirachta indica
A. ANOVA	: Analysis of variance
O. sanctum	: Ocimum sanctum
M. elelngi	: Mimusops elelngi
T. cardifolia	: Tinospora cardifolia
P. umbellate	: Pothomorphe umbellate
A. muricata	: Annona muricata

M. citrifolia	: Morinda citrifolia
EDTA	: Ethyle di amine tetra acetic acid
g	: Gram
mg	: Milligram
ml	: Milliliter
mm	: Millimeter
p- value	: Probability value
sig	: Significant
NS	: Non Significant
BHI broth	: Brain Heart Infusion broth
CEJ	: Cemento enamel junction
CFU	: Colony forming unit
µm	: Micro meter
µg	: Micro gram

INTRODUCTION

One of the very important goal of root canal treatment is the stamping out of microorganisms from root canal system.[1] During endodontic treatment, number of microorganisms within the root canals is reduced as much as possible using mechanical and chemical procedure. However, there is a possibility that some of them are left in the canal. That is why various medications are placed inside the canals during the time period between treatment sessions.[2] The need for medications increases, especially in those cases where an infection is resistant to regular treatment and the therapy cannot be successfully completed due to the presence of pain or continuing exudates.[1]

Persistent endodontic infections are mainly due to retention of microorganism in the dentinal tubules. Enterococcus faecalis is the primary organism detected in persistent asymptomatic infections. Enterococcus faecalis is a facultative anaerobic gram positive rod which can invade the dentinal tubules endure prolonged periods of starvation and possess certain virulence factors and lytic enzymes.[3]

The most effective method for eliminating E. faecalis from the root canal space and dentinal tubules is the use of Sodium hypochlorite and 2% Chlorhexidine. Due to the disadvantages of sodium hypochlorite like unpleasant taste, toxicity and potential weakening of the tooth structure by decreasing the hardness and the structural integrity of the dentine with in the root canal.[4] Owing to the potential

side effects, safety concerns and ineffectiveness of conventional allopathic formulation, consumption of preparations from medicinal plants has increased over the last few decades.[5]

Irrigants not only are important for the removal of debris and dentinal chips produced during cleaning and shaping, but are of clinical importance in the eradication of the radicular infection.[6,7]

Another widely accepted irrigant is 2% Chlorhexidine digluconate. It has a broad spectrum antimicrobial action, low toxicity and property of substantivity, but it cannot dissolve the organic substrate and necrotic tissue from the root canal system. Allergic reactions have also been reported against 2% CHX such as contact dermatitis, desquamative gingivitis, discolouration of the teeth and tongue and dysgeusia.[8]

The constant increase in antimicrobial resistance and side effects caused by synthetic drugs has prompted researchers to look for herbal alternatives. In recent years there is an exponential growth in the field of herbal medicine because of their natural origin, easy availability, efficacy, safety and less side effects.[9]

Studies confirm that the growth of gram +ve and gram -ve food borne bacteria, yeast and moulds can be inhibited by Garlic (Allium sativum), Bitter Guard (Momordica charantia), neem (Azadirichta Indica), Clove (Syzgium aromaticum) and other herbal extractes.

Bitter guard has been used for centuries and has a good source of vitamin C, Vitamin A, phosphorus and iron and has been extensively used in folk medicine. It also had antibacterial activity against E.coli, Pseudomonas, Klebsiella, Bacillus subtilus Staphylococcus, Salmonella, Streptococcus, E. faecalis, Entamoeba histolytica.[10]

Garlic is one of the greatest health tonics and has proven medicinal properties. It contains a substance called 'Allicin' which is equivalent to that of penicillin (1mg of allicin is equated to that of 15 IU of penicillin).[11] Allicin can destroy cell wall and cell membrane of root canal bacteria. Garlic inhibit the growth of oral pathogens such as Streptococcus mutans and P. gingivalis and hence used in the management of dental infections such as periodontitis.[12, 13] It has also been found effective against also against E.faecalis.[14]

This study comparatively evaluate the antimicrobial efficacy of two herbal extracts Bitter Guard (Momordica charantia) and Garlic (Allium sativum) as endodontic irrigants in comparison with NaOCl and Chlorhexidine against E. faecalis.

AIM AND OBJECTIVES

Aim:

To evaluate the antimicrobial efficacy of two herbal extracts i.e, Bitter Guard (Momordica charantia) and Garlic (Allium sativum) as endodontic irrigants against Enterococcus faecalis.

OBJECTIVES:

1. To evaluate the antimicrobial efficacy of two different herbal extracts, Bitter Guard (Momordica charantia) and Garlic (Allium sativum) to eradicate E.faecalis
2. To compare these two herbal extracts, Bitter Guard (Momordica charantia) and Garlic (Allium sativum) efficacy against known endodontic irrigants 5.25% NaOCl and 2% Chlorhexideine.

REVIEW OF LITERATURE

Hernandez M M et al (1999)[15] conducted a study on the biological activities of crude plant extract from Vitex trifolia L. the hexanic extract from leaves completely inhibited the growth of fungal plant pathogen Fusarium sp. with in the first 2 days of experiment, but dropped significantly at day 6.

Gomes BPFA et al (2003)[1] conducted in vitro study to evaluate the effectiveness of 2% chlorhexidine gel and calcium hydroxide against E. faecalis. They concluded that 2% chlorhexidine gel alone was more effective against E. faecalis than $Ca(OH)_2$.

Nageswar rao et al (2004)[16] conducted a study to evaluate the efficacy of an intra canal medicament comprising of calcium hydroxide and 2% chlorhexidine against E. faecalis. The results showed that the paste made from calcium hydroxide and 2% chlorhexidine was significantly more effective than that made from alone calcium hydroxide and 2% chlorhexidine.

Valera MC et al (2010)[17] evaluated the antimicrobial activity of 2% chlorhexidine gel associated with various intracanal medicaments against Candida albicans and Enterococcus faecalis inoculated in root canals. They concluded that the use of 2% chlorherixidine gel reduces the number of microorganisms significantly; only the calcium hydroxide and calcium hydroxide associated with chlorhexidine are able to eliminate these microorganisms completely.

Kannathasan et al (2011)[18] conducted a study to check the antibacterial activity of leaf methanol extracts of five different species of Vitex. The results of antibacterial activity of vitex species showed that the extracts possessed a broad spectrum of antibacterial activity against all microorganism screened in the study.

Ahangari Z et al (2012)[2] done a study to assess the antimicrobial activity of propolis in comparison with $Ca(OH)_2$ against E. faecalis and concluded that the antimicrobial activity of propolis against E. faecalis was comparible with $Ca(OH)_2$ at different time intervals and thus can be used as an alternative intra canal medicament.

Ehsani M et al (2013)[19] compare the antibacterial activity of Chlorhexidine with two natural drugs. The antibacterial activities of three different propolis extracts (alcohol concentrations: 0, 15, 40%) and Aloe vera gel on E. faecalis were compared. The results of the study showed the hydroalcoholic extracts of propolis and Aloe vera gel had antibacterial effects on E. faecalis. However, propolis is more potent than A. vera. They concluded that appropriate concentrations of alcoholic extracts of propolis and some fractions of A. vera gel might be good choices for disinfecting the root canal in endodontic treatments.

Valera MC et al (2013)[20] evaluated the antimicrobial activity of auxiliary chemical substances and natural extracts on Candida albicans and Enterococcus faecalis inoculated in root canals. They concluded that 2.5% sodium hypochlorite and 2% chlorhexidine gel were more

effective in eliminating C. albicans and E. faecalis, followed by the castor oil and glycolic ginger extract. The Aloe vera extract showed no antimicrobial activity.

Maekawa LE et al (2013)[21] evaluated the effectiveness of glycolic propolis and ginger extracts, calcium hydroxide, chlorhexidine gel and their combinations as intra canal medicaments against Candida albicans, Enterococcus faecalis, Escherichia coli and endotoxins in root canals. They concluded that all intra canal medicaments were able to eliminate the microorganisms in the root canals and reduce their amount of endotoxins; however, calcium hydroxide was more effective in neutralizing endotoxins and less effective against C. albicans and E. faecalis, requiring the use of medication combinations to obtain higher success.

Ghonmode WN et al (2013)[22] done a study on antimicrobial efficacy of herbal alternatives as endodontic irrigants was evaluated and compared with the standard irrigant sodium hypochlorite. They concluded that neem leaf extract has a significant antimicrobial effect against E. faecalis. Microbial inhibition potential of neem leaf extract observed in this study opens perspectives for its use as an intracanal medication.

Kumar H (2013)[23] evaluated the antimicrobial efficacy of Curcuma longa, Tachyspermum ammi, chlorhexidine gluconate gel and calcium hydroxide as intracanal medicaments against Enterococcus

faecalis. Author concluded that Curcuma longa can be used as intracanal medicament in endodontic failure cases.

Castilho AL et al (2013)[24] evaluated 25 plant extracts obtained from Brazilian forests against planktonic E. faecalis and were subjected to two traditional antibacterial assays, the micro dilution broth assay and the disk diffusion assay, using chlorhexidine as a control. The results of the study discovered six active extracts against planktonic E. faecalis and support further testing via assays involving biofilm formation, as well as the determination of the compound chemical profiles, as their activity was significantly better than that observed for chlorhexidine.

Rosaline et al (2013)[25] done a study to assess the antibacterial efficacy of three different herbal irrigants Morinda citrifolia, Azadirachta indica, Green tea against E. faecalis. Results of the study showed that Azadirachta indica produced maximum reduction in adherence to dentine followed by NaOCl, Green tea and Morinda citrifolia. They concluded that neem is effective in preventing adhesion of E. faecalis to dentine.

A. Gupta et al (2013)[26] evaluated the antimicrobial efficacy of Ocimum santum, Cinnamomum zeylanicum, Syzygium aromaticum and 3% NaOCl against E. faecalis in planktonic suspension and biofilm phenotypes. They concluded that Cinnamomum zeylanicum, Syzygium aromaticum and Ocimum santum demonstrated antimicrobial activity

against planktonic and biofilm forms of E. faecalis with Cinnamomum zeylanicum and Syzygium aromaticum having better antimicrobial efficacy than Ocimum santum. NaOCl had superior antimicrobial efficacy amongst all the groups.

Vinothkumar et al (2013)[27] evaluated the efficacy of various herbal extracts Cucuma longa, Azadirachta indica, Aloe vera, Myristica fragrans and terminalia chebula as endodontic irrigant against E. faecalis and Candida albicans using real time quantitative polymerase chain reaction. Results of the study showed that neem was highly efficient to 5.25% NaOCl in reducing E. faecalis and C. albicans with in root canals when compared with other extracts.

Valera MC et al (2014)[28] evaluated the biomechanical preparation action on microorganisms and endotoxins by using sodium hypochlorite and an intracanal medication containing Zingiber officinale, with or without calcium hydroxide. The results showed that the NaOCl eliminated 100% of root canal microorganisms and reduced 88.8% of endotoxins immediately after biomechanical preparation, and 83.2% at 7 days after biomechanical preparation.

Mistry KS et al (2014)[29] checked the antimicrobial activity of Azadirachta indica (Neem), Ocimum sanctum (Tulsi), Mimusops elelngi (Bakul), Tinospora cardifolia (Giloy) and Chlorhexidine Gluconate (CHX) on common endodontic pathogens like Streptococcus mutans, Enterococcus faecalis and Staphylococcus aureus. They concluded that Methanolic extract of A. Indica, O.sanctum, M. Elengi,

T. cardifolia and Chlorhexidine Gluconate has considerable antimicrobial activity against S. mutans, E. faecalis and S. aureus.

Sponchiado EC et al (2014)[30] done a study to assess the antimicrobial activity of an intracanal medication containing the ethyl-acetate fraction of Pothomorphe umbellata against Enterococcus faecalis. They concluded that Ethyl-acetate fraction of P. umbellate was efficient against E. faecalis, making this phytotherapy a viable option for endodontic treatment.

Birring OJ et al (2015)[31] done a study to assess the anti-microbial efficacy of garlic extract against Enterococcus faecalis biofilm and its ability to penetrate into root dentin. The results indicate that Garlic has a potential to serve as an alternative herbal root canal irrigant being an effective and biocompatible anti-microbial agent with good dentinal penetration property.

Karkare SR et al (2015)[32] compared the antimicrobial activity of saturated and diluted (1:1) hydroalcoholic extract of Aloe vera, garlic, and 5% NaOCl against E. faecalis using the commonly used agar diffusion method. The results of the study showed saturated hydroalcoholic extract of A. vera showed the highest zone of inhibition against E. faecalis. NaOCl, which is considered as gold standard, also showed higher zones of inhibition.

Mathew J et al (2015)[33] assessed the effectiveness of an indigenously prepared herbal extract "EndoPam" and compare it with

the conventional endodontic irrigants for disinfection of root canals infected with Enterococcus faecalis. The results of the study showed that in the preliminary Agar diffusion study, EndoPam exhibited a zone of inhibition comparable to that of sodium hypochrorite. The diameter of the inhibition zone was in the following order: 2% chlorhexidine gluconate > EndoPam > 5.25% NaOCl > Normal Saline. The qualitative assay done by culturing the bacteria after a period of 3 weeks showed no bacterial growth in any of the tested irrigants, except in normal saline.

Saxena D et al (2015)[34] evaluated and compare the antimicrobial activity of five herbal extracts, i.e., Propolis, A. indica, Triphala, C. longa, and MC with that of 2.5% sodium hypochlorite against Enterococcus faecalis. Results of study showed Propolis showed highest zone of inhibition among all the herbal extracts next to sodium hypochlorite. They concluded that Propolis and A. indica have significant antimicrobial activity against E. faecalis.

Chandrappa PM et al (2015)[35] assessed the antimicrobial activity of herbal medicines tulasi extract and neem extract and chlorhexidine against E. Faecalis. They concluded that both 2 herbal extracts showed significant inhibitory effect against E. Faecalis compared to 2% chlorhexidine. Thus these can be used as alternatively as endodontic irrigants or medication.

N. Radwan et al (2015)[36] evaluated the antimicrobial efficacy of medicinal plant extracts neem leaf extract, Ginger extract, Miswak

extract, lemon solution, when used as root canal irrigant on E. faecalis. They concluded that medicinal herbs may offer new source of antibacterial agents for use and might be used in the development of a promising irrigants, which might be safer than other chemical compounds used in the endodontic treatment process.

Zeenath Ambareen et al (2015)[37] done a study to evaluate and compare extracts of ginger, garlic aloe vera, neem, turmeric and NaOCl as root canal irrigants. The results of the showed that mean zone of inhibition was recorded in NaOCl followed by garlic, neem, ginger and turmeric extract. Lowest mean zone of inhibition was found in Aloe vera extract.

Mustafa M et al (2016)[38] done a study to assess the antimicrobial efficacy of neem (Azadirachta indica) extract against Enterococcus faecalis. They concluded that neem leaf extract shows comparable zones of inhibition with that of chlorhexidine and sodium hypochlorite. Neem leaf extract has significant antimicrobial activity against E. faecalis and thus opens the perspectives for the use of neem extract as an intracanal medication.

Valera MC et al (2016)[39] evaluated the antimicrobial activity of 2% chlorhexidine gel (CHX) as auxiliary chemical substance and intracanal medications on Candida albicans, Enterococcus faecalis, Escherichia coli, and their endotoxins in the root canals. They concluded that the instrumentation using CHX and intracanal

medication used were able to eliminate the microorganisms from the root canal; the endotoxins were reduced, yet not completely eliminated.

Mathew J et al (2016)[40] done a study to determine the antimicrobial effect of water extracts of leaves of Annona muricata and Simarouba glauca on Enterococcus faecalis using agar diffusion method. Results showed that the leaf extract of A. muricata showed similar effectiveness as that of sodium hypochlorite, whereas the leaf extract of S. glauca showed only a slight reduction in growth of E. faecalis. They concluded that Leaf extract of A. muricata can be developed as an alternative to sodium hypochlorite for root canal irrigants.

Shenoi PR et al (2016)[41] compared the antimicrobial efficacy of BioPure MTAD, 0.2% chitosan, 1% chitosan, 2% chlorhexidine gluconate, and 3% sodium hypochlorite against Enterococcus faecalis, which is frequently isolated from persistent root canal infections. They concluded that 1% chitosan can be an effective natural antimicrobial substitute for synthetic irrigants.

Jose J et al (2016)[42] compared the antimicrobial efficacy of different irrigants like QMiX, guava leaf extract, alo evera extract, 2.5% sodium hypochlorite and 2% chlorhexidine gluconate against Enterococcus faecalis and Candida albicans. They concluded that Guava leaf extract showed significant inhibitory effects against Enterococcus faecalis and Candida albicans. QMiX demonstrated the

best results among the tested solutions and can be considered as a potential alternative to existing root canal irrigants.

Babaji P et al (2016)[43] evaluated the antimicrobial effect of herbal root canal irrigants Morinda citrifolia, Azadirachta indica extract, Aloe vera with sodium hypochlorite. The results of the study showed that highest inhibitory zone against E. faecalis was seen in Naocl followed by M. citrifolia and A. indica extract and the least by A. vera extract.

Tonea A et al (2017)[44] done a study focuses on the comparison of the antibacterial and antifungal properties of different endodontic products, two commercially available, one experimental plant based extract, and two control substances. They concluded that the experimental mix extract of Arctium lappa root powder and Aloe vera gel is able to inhibit very resistant microorganisms, like Enterococcus faecalis and Candida albicans.

Nourzadeh M et al (2017)[45] evaluated the antimicrobial effect of Eucalyptusgalbie and Myrtus communis L. methanolic extracts, chlorhexidine and sodium hypochlorite on E. Faecalis as the predominant species isolated from infected root canals. They concluded that although 5.25% NaOCl was the most effective irrigant, all agents exerted acceptable antimicrobial activity against E. faecalis.

Yadav P et al (2017)[46] evaluated the cytotoxic effect and antibacterial efficacy of chitosan when used as root canal irrigant against E. Faecalis and Candida albicans biofilm formed on tooth

substrate. They concluded that the use of chitosan as a root canal irrigant might be an alternative considering the various undesirable properties of NaOCl and chlorhexidine.

Ehsani M et al (2017)[47] compared the antibacterial activity of Chlorhexidine with two natural drugs propolis extracts and A. vera gel on E. faecalis. They concluded that appropriate concentrations of alcoholic extracts of propolis and some fractions of A. vera gel might be good choices for disinfecting the root canal in endodontic treatments.

MATERIALS AND METHODS

MATERIALS :

- 50 extracted human mandibular premolars teeth
- Diamond disc
- Straight hand piece (NSK EX - 6) S.No: F6X44766; Japan
- Micro-motor NSK EBB75900
- Size 10 K-files (Mani Inc, Japan)
- Sizes 15 K-files (Mani Inc, Japan)
- Size 40 H file(Mani Inc, Japan)
- Protaper universal rotary files SX, S1, S2,F1,F2,F3 – 21mm (Dentsply Maillefer)
- EndoMate DT NSK model MPFI6R C871001
- Mini Endo Block (Dentsply Maillefer, Ballaigues)
- 5.25% Sodium HypoChlorite (Comdent corporation, Mumbai)
- 2% Chlorhexidine
- 17% EDTA (Pulpdent Corporation USA)
- Normal Saline (Baxter, Tamilnadu,)
- Cyanoacrylate glue
- BHI broth
- Distilled waster
- Ethanol
- Methanol
- Muslin cloth
- Whatman no. 1 filter paper

- Bitter guard extract
- Garlic extract
- Test tubes
- Micro pippetes
- Agar plates

METHODOLOGY:

Selection of teeth and canal preparation:

Fifty Single rooted human mandibular premolar extracted for orthodontic reasons. The teeth with extremely curved roots, fracture lines, severely calcified roots and root caries were excluded from the study. The teeth selected had single canal with straight roots measuring approximately 21mm. In the first step, the anatomical crown of all the teeth were decoranated at Cemento enamel junction (CEJ) perpendicular to the long axis of the teeth using a diamond wheel bur and micro motar. The remaining roots measured 12-15mm. The exploration of the radicular canal was accomplished with no 10 and no 15K file to make sure that the roots had only one canal and it was patent. Then the working length was determined one mm short of the file penetration into the canal. Cleaning and shaping of root canals were done by crown down technique using Protaper universal rotary files till F3. The canals were recapitulated and irrigated with 5.25% NaOCl, 17% EDTA and final rinse was with normal saline. Subsequent to the canal preparation the apical foramen of all the specimens were sealed with cyanoacrylate glue to prevent bacterial microleakage.

Specimens were placed in steel containers containing BHI broth and subjected to autoclave at 121°c at 15psi for 20 minutes for sterilization. Subsequent to sterilization all the specimens were transported and manipulated under laminar flow using sterile instruments and equipments.

Preparation of E. faecalis suspension and tooth inoculation:

In order to get a controlled and standard suspension of the organism the following procedure was adopted. From a stock culture of MTCC 2527 E. faecalis strain, subculture was made onto a plate of Diagnostic Sensitivity Test Agar. From this a typical colony was sub-cultured into 50 ml of Streptococcus Selection Broth contained in a 100 ml conical flask. This was incubated at 37° C for 24 hours. Enumeration of live bacteria (CFU) was carried by serial dilution method. For injecting into the tooth a suspension of bacteria containing 10µg CFU per ml was used. The root canals were inoculated with E. Faecalis suspension using sterile 1ml tuberculin syringes and specimens were separately placed in steel containers containing 2ml of broth. The steel containers containing the specimens were kept in incubators at 37° c for 21 days.

Preparation of Extract:

Bitter Guard (Momordica charantia): The ripe and unripe fruits of M. charantia were obtained from local market. Fruits were washed with distilled water, and the seeds were separated. The fruits were then

sliced into small pieces and dried in drying oven at 50^0C. The dried plant materials were then blended into powder using an electric blender for extraction. The powdered seeds and fruits of M. charantia were separately extracted with ethanol by using Soxhlet apparatus for 24hours. The extract was concentrated using a rotary evaporator.[48]

Garlic (Allium sativum) used in the present study were obtained from local market. The bulbs were peeled and washed with distilled water. The bulbs were squeezed and were sucked in methanol for 8hours with 10 minutes interval shaking. The extract was filtered using muslin cloth followed by filter paper. The filtrate was evaporated at 45^0C to dryness and the dried substance was kept in sterile bottle under refrigerated condition until use.[49]

Antimicrobial assessment:

After 21 days all the specimens were retrieved and each specimen was transferred into test tubes containing 3ml of saline and was shaken three times for 30 seconds each time on a rotator to remove the excess culture medium. In addition large amount of bacteria present on the surface of the specimen were removed during rinsing and irrigation. The samples were divided into five groups, each containing ten teeth. Test irrigating solutions were used as follows.

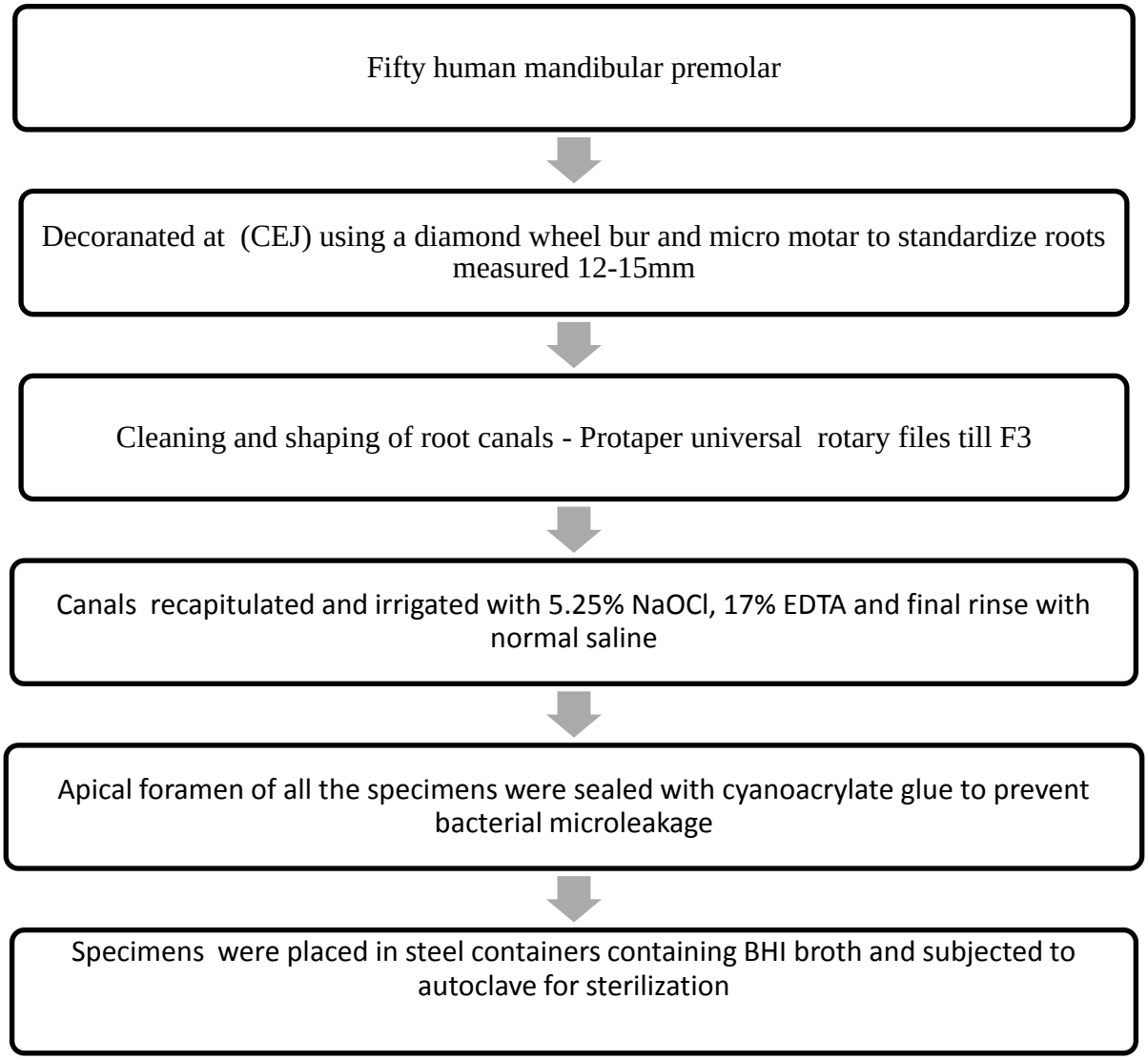
GROUPS n=10	TEST SOLUTION
Group 1	Normal Saline
Group 2	5.25% NaOCl
Group 3	2% CHX
Group 4	Bitter guard
Group 5	Garlic

Samples in each were irrigated with respective irrigating solutions using 2 ml syringes and were immersed in test tubes containing 2ml of the solution for 5minutes. Subsequent to the removal of specimens from the test tubes each specimen was transferred into test tube containing 3ml of saline and shaken in a rotator for 3 times for 30 second each.

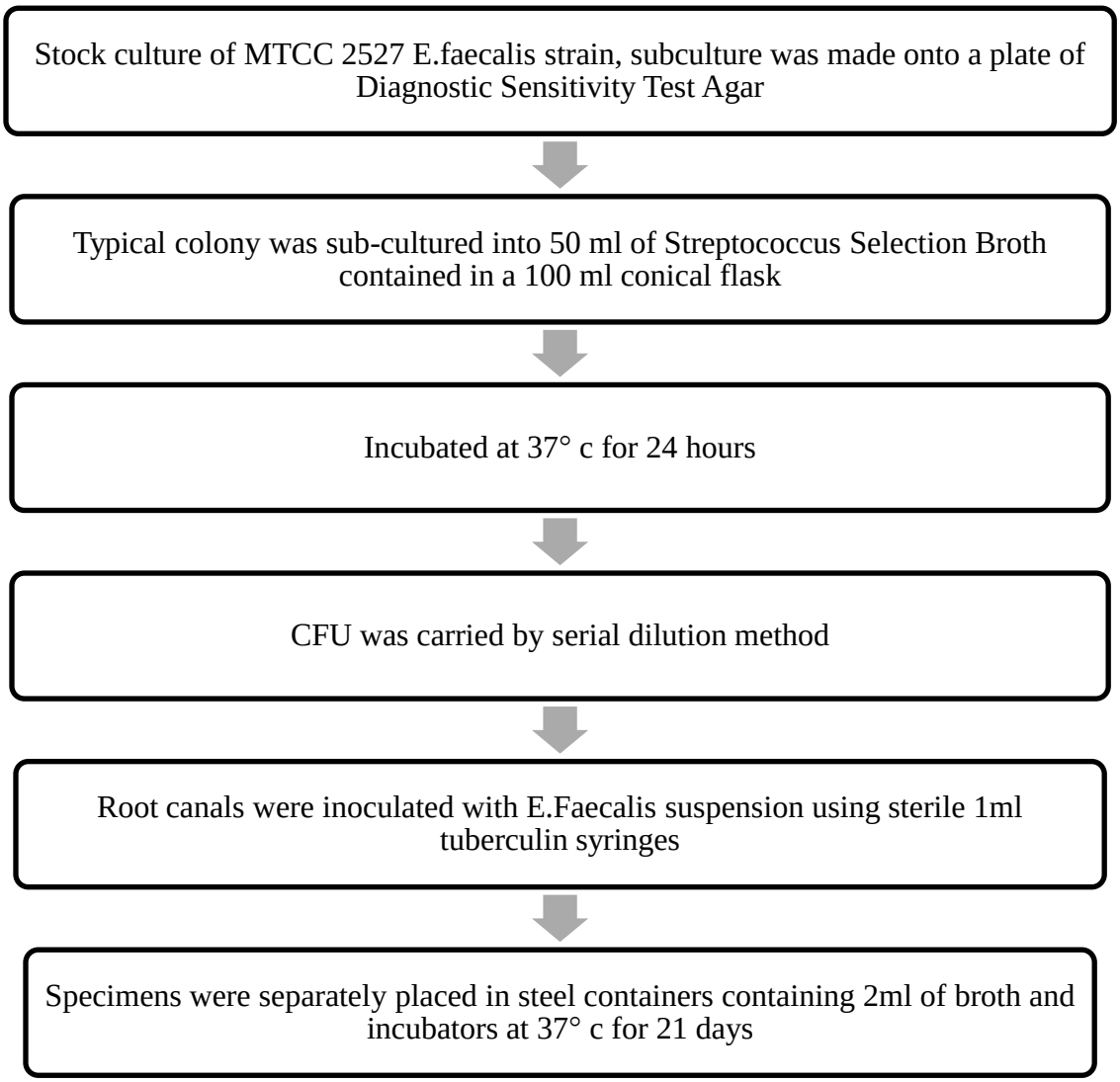
Dentinal shavings were collected using no 40 H file in an aseptic condition. Shavings were transferred into test tubes containing 10 ml sterile normal saline . Three serial dilution was carried out i.e., till 10^3. From this one ml was pipetted on to a sterile 100 mm diameter in duplicate. To each of these plates 15 ml of agar medium, melted, cooled and was added mixed well and allowed to solidify. These plates were incubated for 24hours at 37° C. After incubation the number of colonies was counted in suitable plates. The number of the colonies multiplied by the dilution factor gives the total number of CFU in the scrapings per tooth.

FLOW CHART OF METHODOLOGY

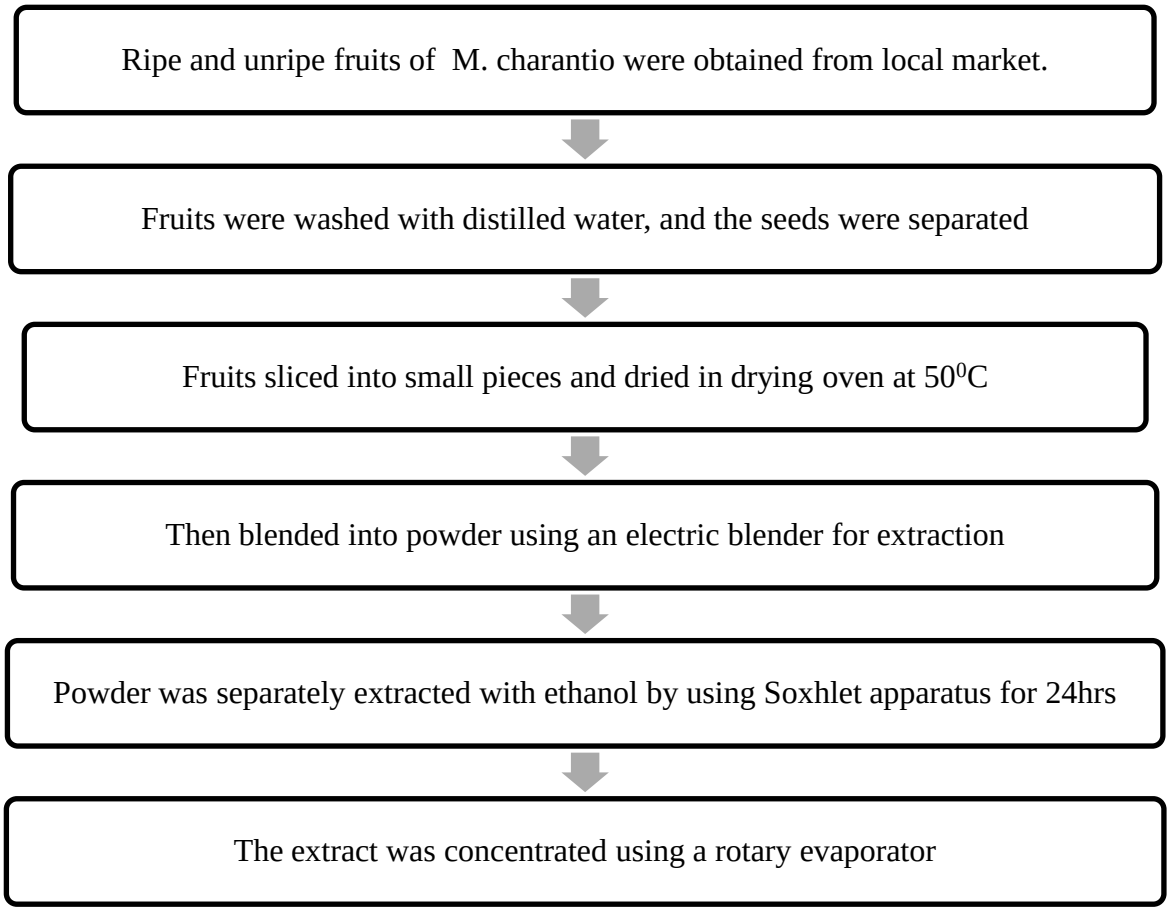
Flow chart for Selection of teeth and canal preparation:



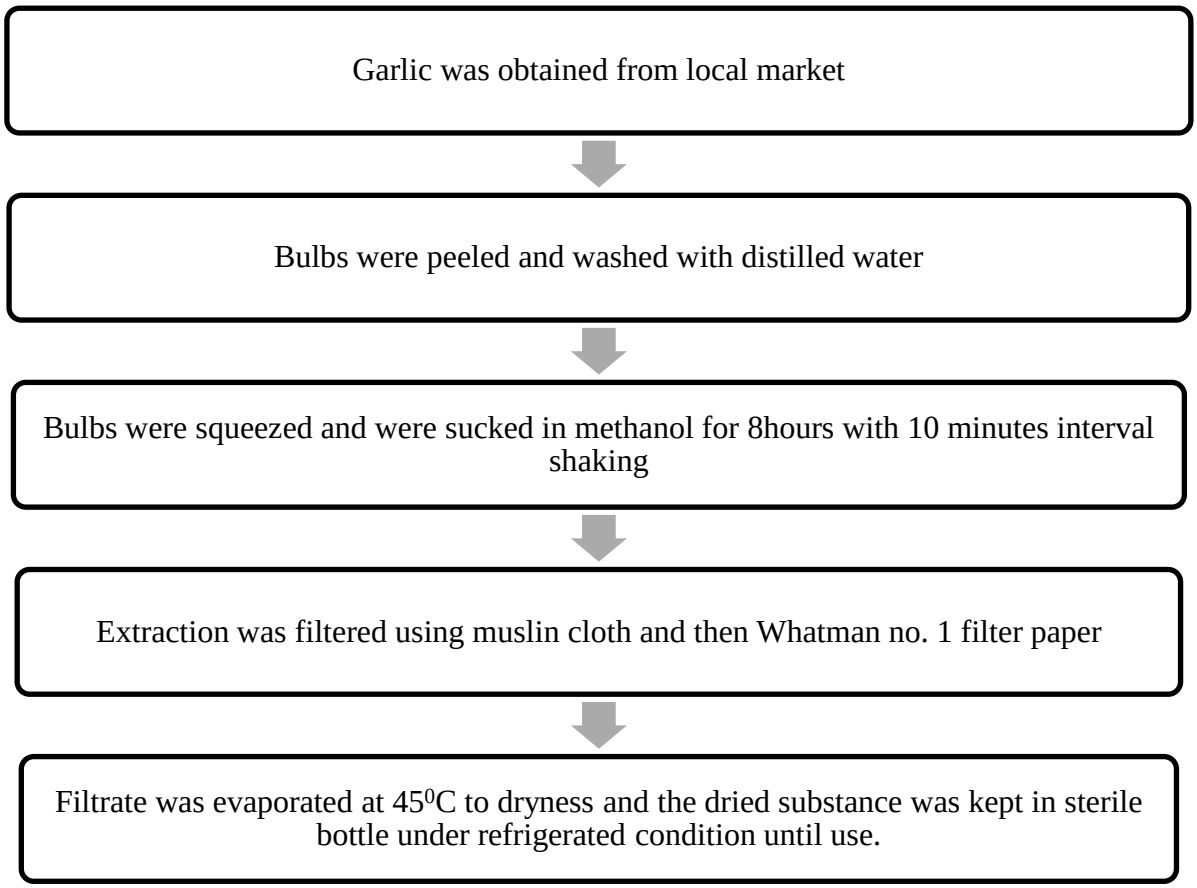
Flow chart for Preparation of E.faecalis suspension and tooth inoculation



Flow chart for Preparation of Bitter Guard (Momordica charantiao) Extract



Flow chart for Preparation of Garlic (Allium sativum) extract



Flow chart for Antimicrobial assessment

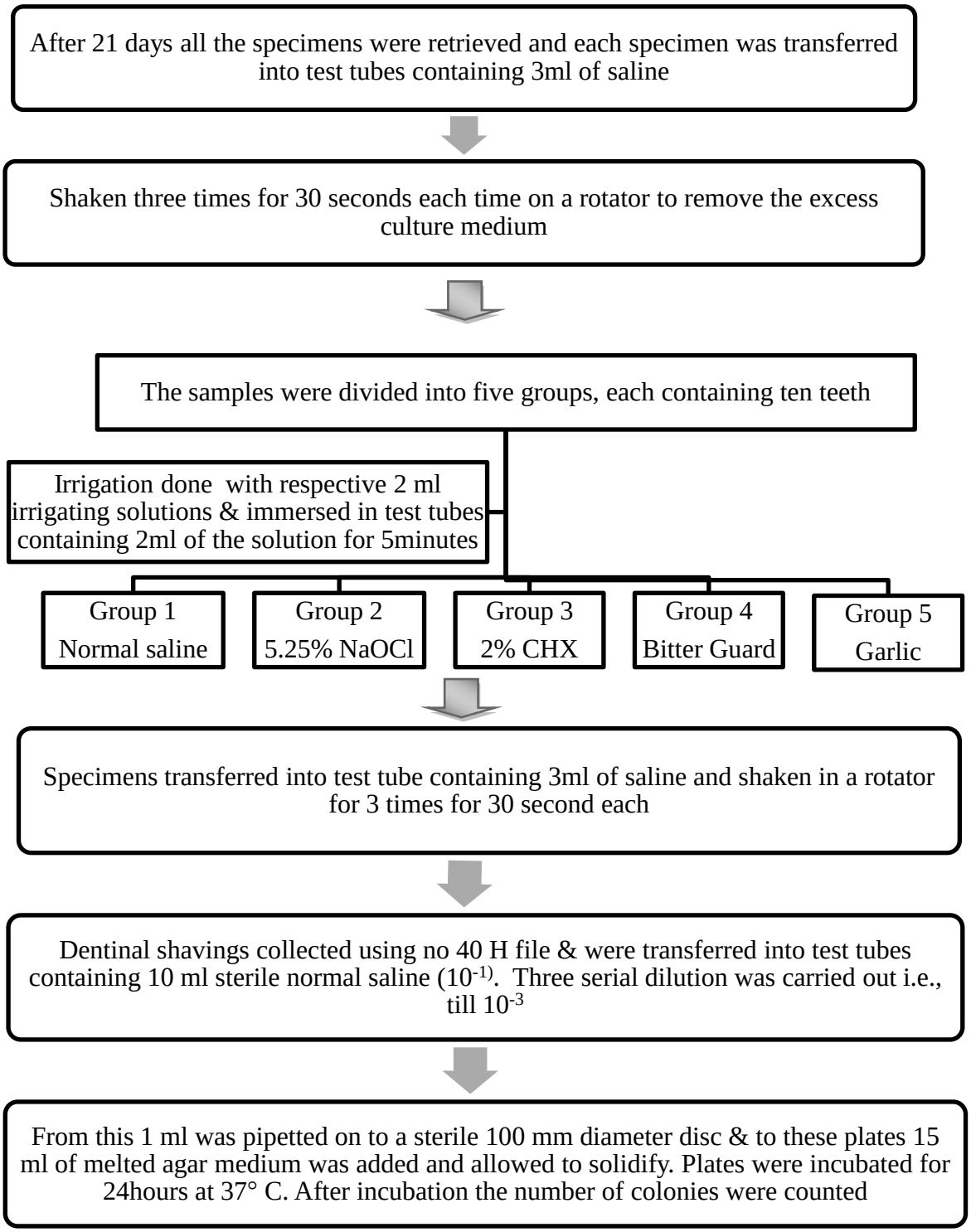


Figure 1: 50 Single rooted extracted mandibular premomolars

Figure 2: Extracted Human Mandibular Premolar Decorated

Figure 3: Armamentarium for cleaning and shaping of root canal

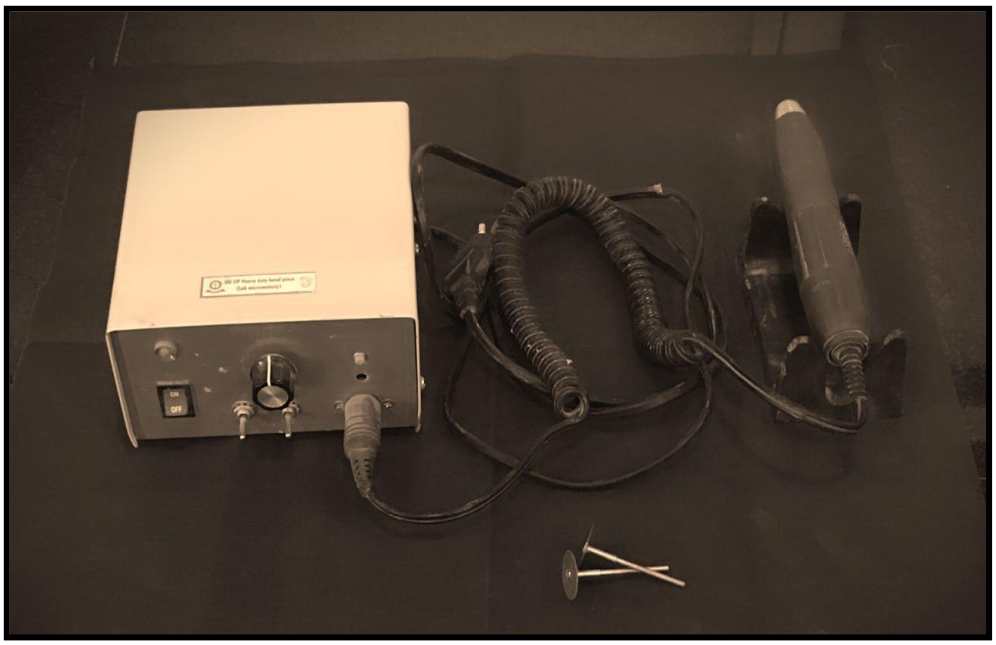


Figure 4: Micro motor for decoronation of teeth

Figure 5: Garlic (Allium sativum)

Figure 6: Preparation of Methanolic Extract of Garlic

Figure 7: Bitter Guard (Momordica charantiao)

Figure 8: Preparation of Ethanolic Extract of Bitter Guard

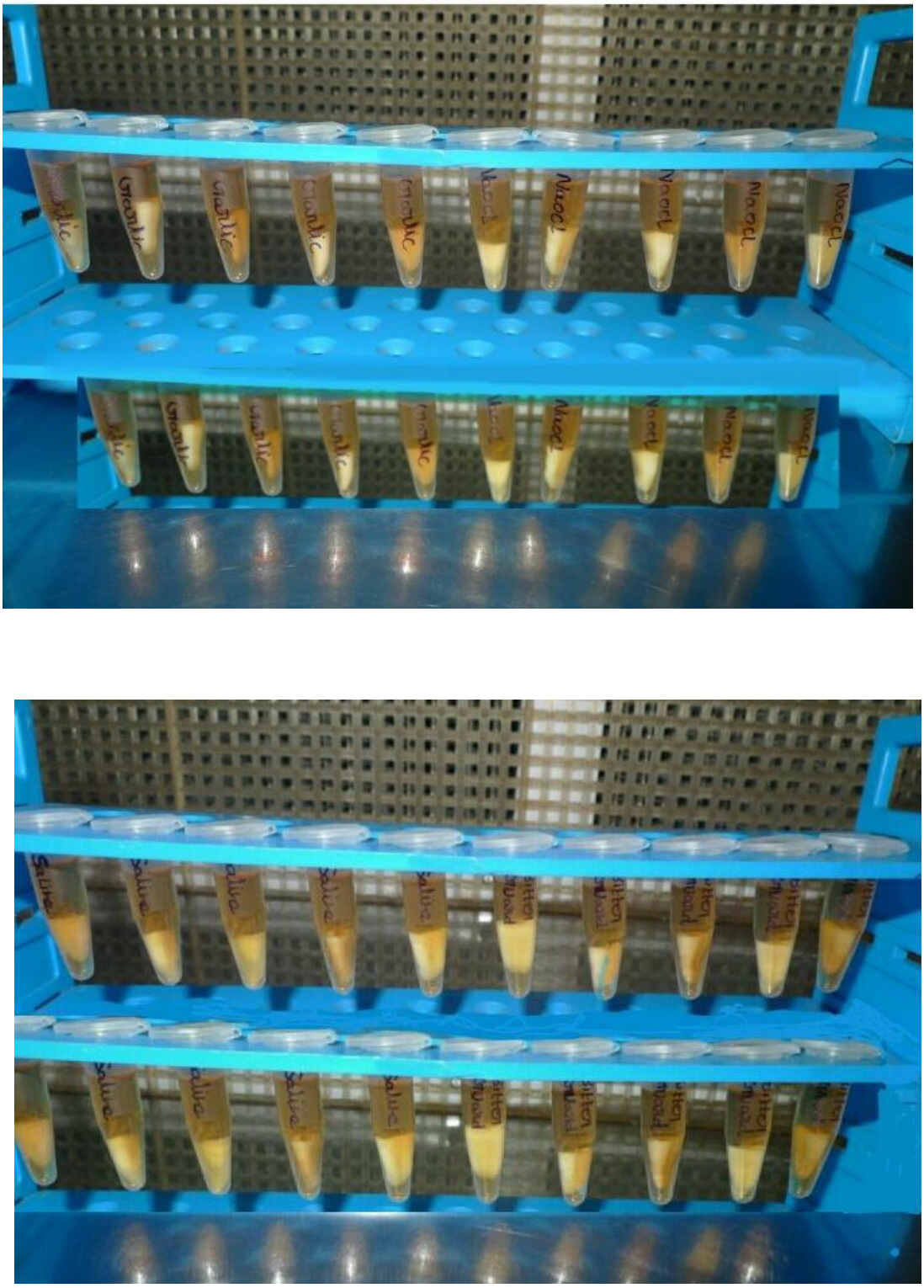


Figure 9: Tooth samples inoculated in E. faecalis Suspention

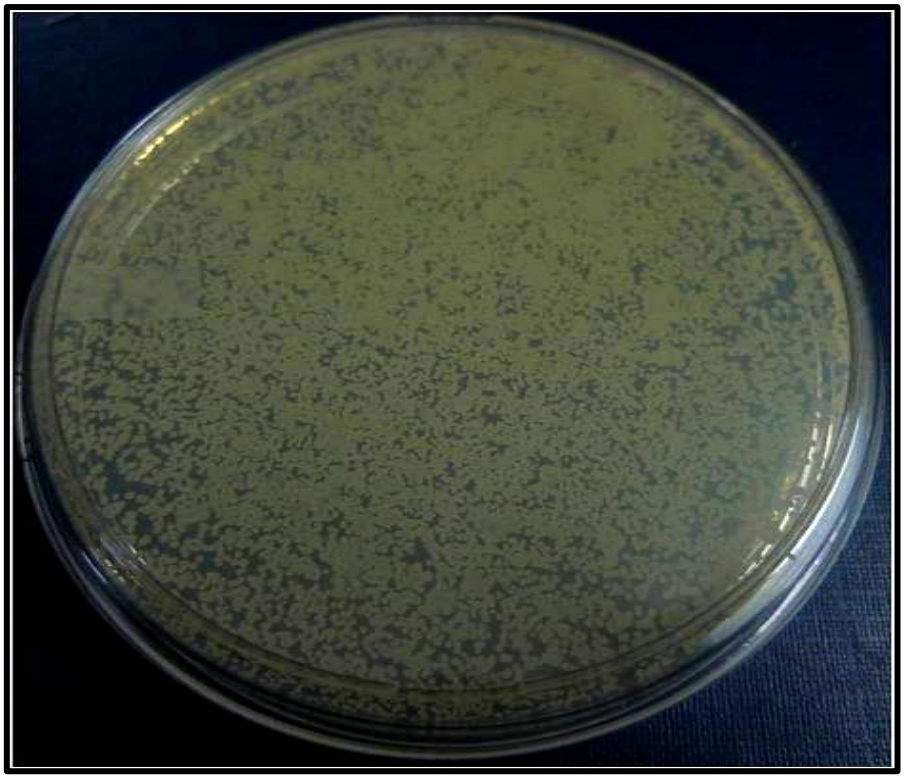


Figure 10: Agar plates showing CFU with normal saline

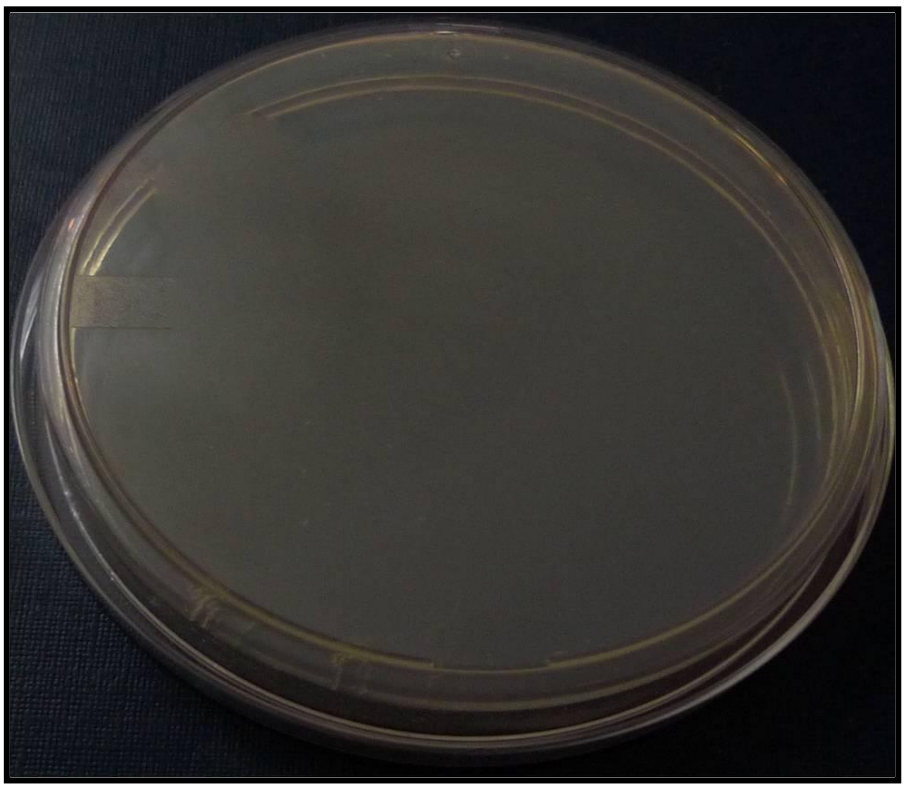


Figure 11: Agar plates showing no CFU with 5.25% NaOCl

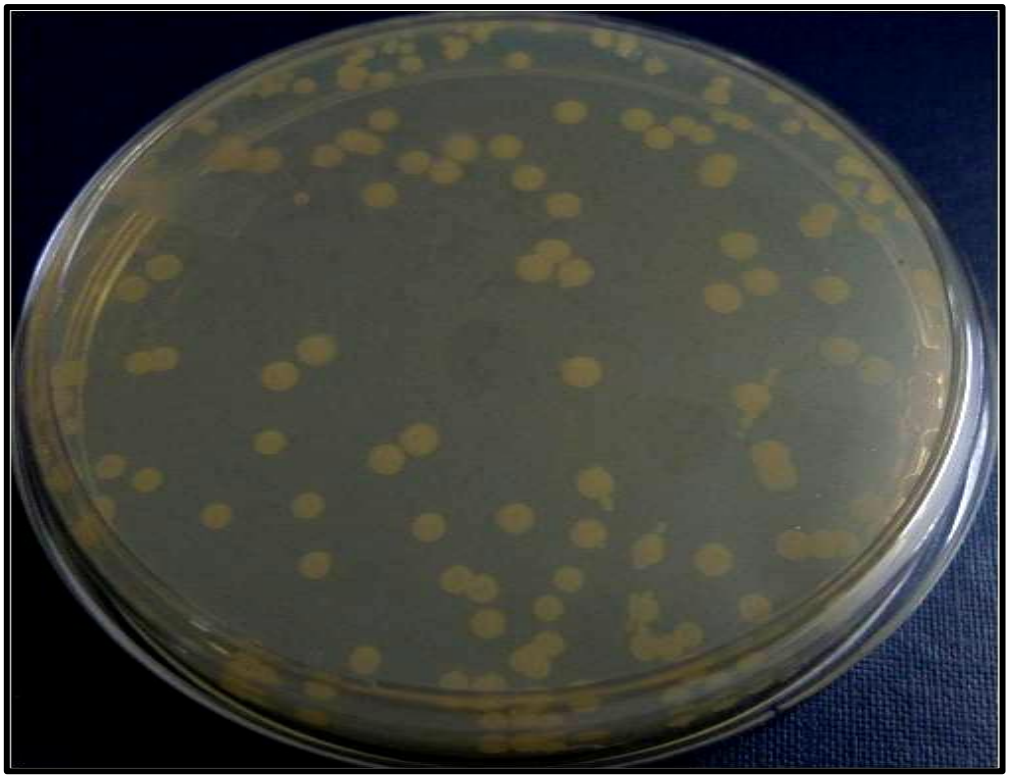


Figure 12: Agar plates showing CFU with 2% CHX

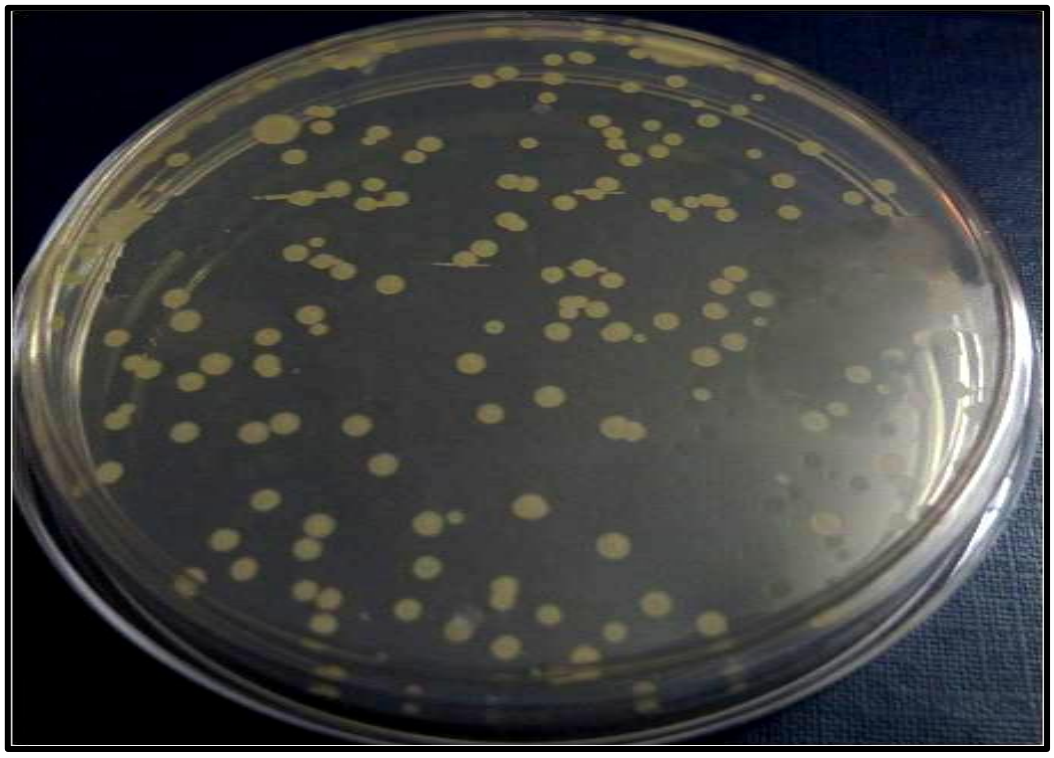


Figure 13: Agar plates showing CFU with Bitter gurad

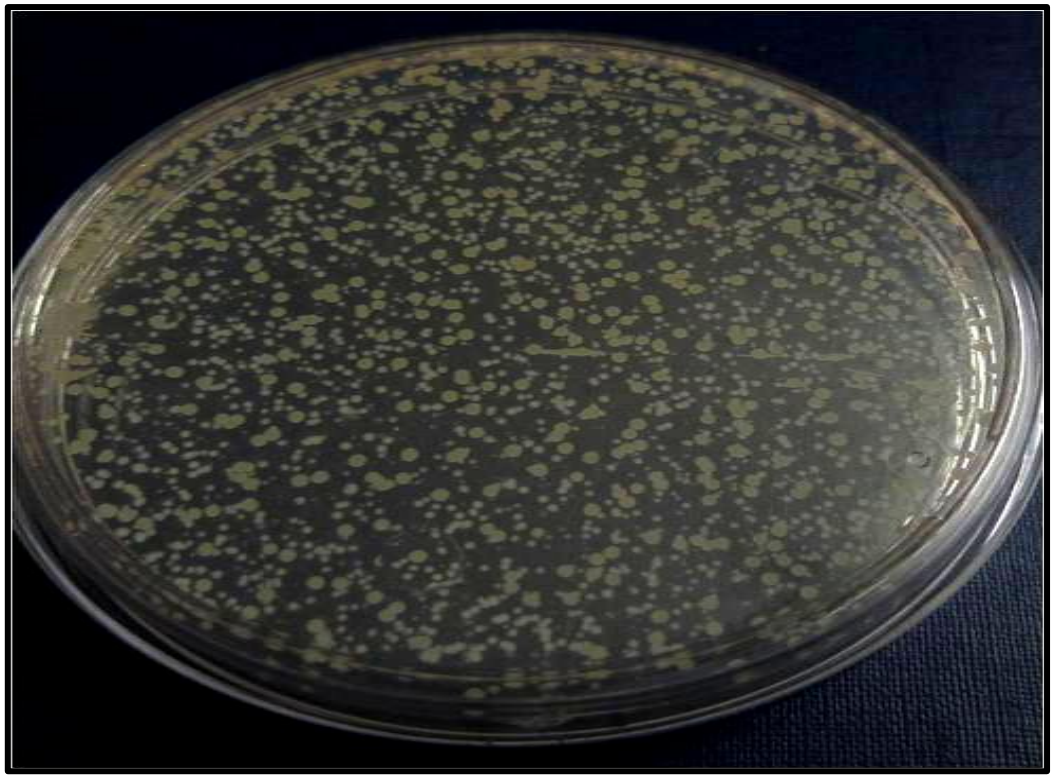


Figure 14: Agar plates showing CFU with Garlic

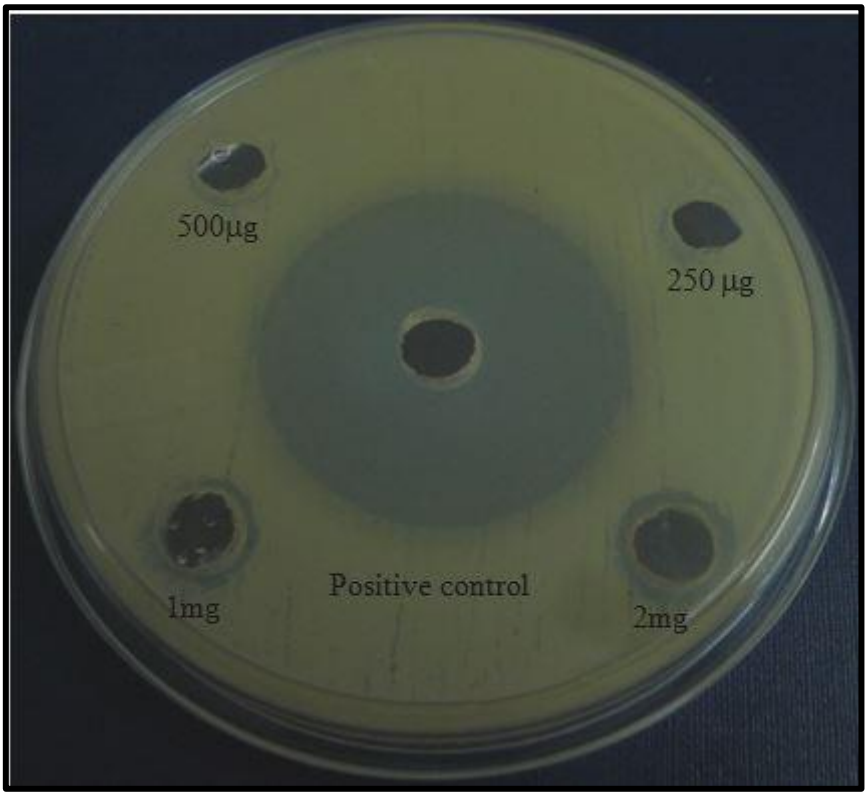


Figure 15: Zone of inhibition with Bitter guard

(12mm with 1mg and 14mm with 2mg)

Figure 16: Zone of inhibition with Garlic

(No visible Zone of inhibition)

RESULTS

From the present in vitro study, following results were obtained

- Test herbal extracts Bitter Guard (Momordica charantia) and Garlic (Allium sativum) are effective against E. faecalis
- 5. 25% NaOCl showed complete inhibition of E. faecalis
- Among the two herbal extract Bitter Guard (Momordica charantia) was more effective than Garlic (Allium sativum), which is nearly equal to 2% Chlorhexidine.
- The means CFU from low to high with all irrigants tested was as follows Group 2 - 5. 25% NaOCl (0.00); Group 3- 2% chlorhexidine (1.14×10^{-3}); Group 4- Bitter Guard (1.40×10^{-3}); Group 5- Garlic (10.30×10^{-3}) and Group 1- Normal saline (28.60×10^{-3}) **(Table 1)**

Table 1: DESCRIPTIVE STATISTICS

Groups	Mean (CFU x 10^{-3}cfu/ml)	Std. Deviation
Group 1	28.60	3.66
Group 2	.00	.00
Group 3	1.15	.16
Group 4	1.40	.18
Group 5	10.30	3.53

INTER GROUP COMPARISON:

Inter group comparison was performed to evaluate and compare the efficacy of 5 different irrigants against E. faecalis **(Graph 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11)** Kruskall wallis ANOVA test showed statistical significance difference between the 5 groups ($p<0.001$) **(Table 2)**

Table 2: COMPARISON BETWEEN FIVE GROUPS

Groups	Median (CFU x 10^{-3}cfu/ml)	IQR	p value
Group 1	30	5.5	<0.001
Group 2	0	0	
Group 3	1.1	0.22	
Group 4	1.4	0.32	
Group 5	11	2.25	

To find out the significant pair and mean difference between the groups Tukey's post hoc test was used. This test was applied for pair wise comparison of the 5 groups to compare effectiveness of irrigants amongst themselves. **(Table 3)**

Table 3: INTER GROUP COMPARISON USING TUKEYS'S POST HOC TEST

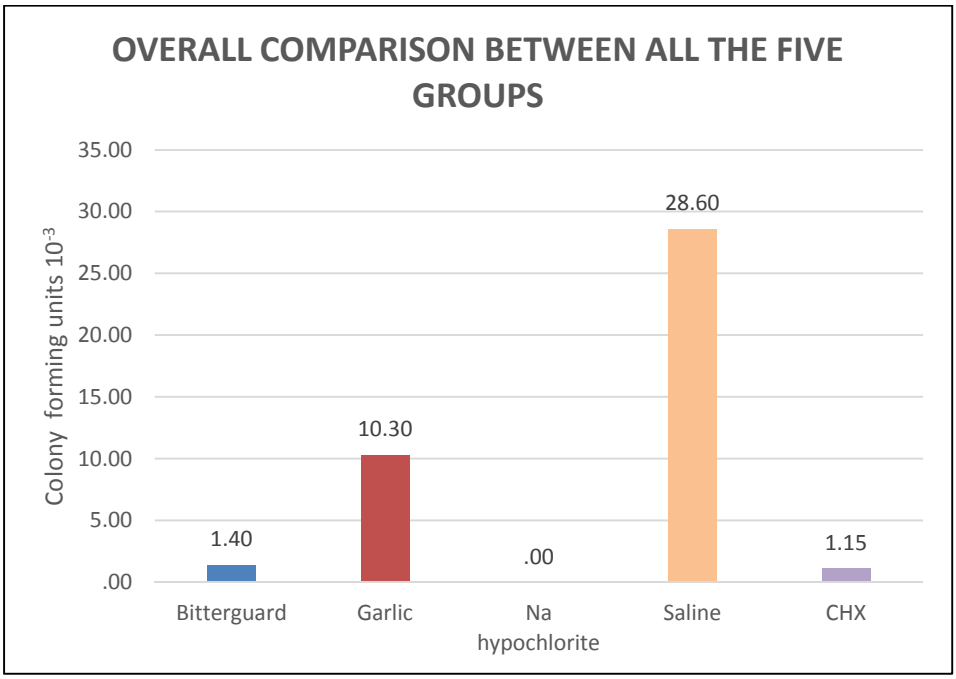
(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	Sig.
Group 1 Normal saline	Bitter guard	27.20000	1.01763	.000*
	Garlic	18.30000	1.01763	.000*
	NaOCl	28.60000	1.01763	.000*
	Chlorhexidine	27.45000	1.01763	.000*
Group 2 NaOCl	Bitter guard	-1.40000	1.01763	.646 (NS)
	Garlic	-10.30000	1.01763	.000*
	Saline	-28.60000	1.01763	.000*
	Chlorhexidine	-1.15000	1.01763	.790(NS)
Group 3 Chlorhexidine	Bitter guard	-.25000	1.01763	.999(NS)
	Garlic	-9.15000	1.01763	.000*
	NaOCl	1.15000	1.01763	.790(NS)
	Saline	-27.45000	1.01763	.000*
Group 4 Bitter Guard	Garlic	-8.90000	1.01763	.000*
	NaOCl	1.40000	1.01763	.646(NS)
	Saline	-27.20000	1.01763	.000*
	Chlorhexidine	.25000	1.01763	.999(NS)
Group 5 Garlic	Bitter guard	8.90000	1.01763	.000*
	NaOCl	10.30000	1.01763	.000*
	Saline	-18.30000	1.01763	.000*
	Chlorhexidine	9.15000	1.01763	.000*

P<0.05

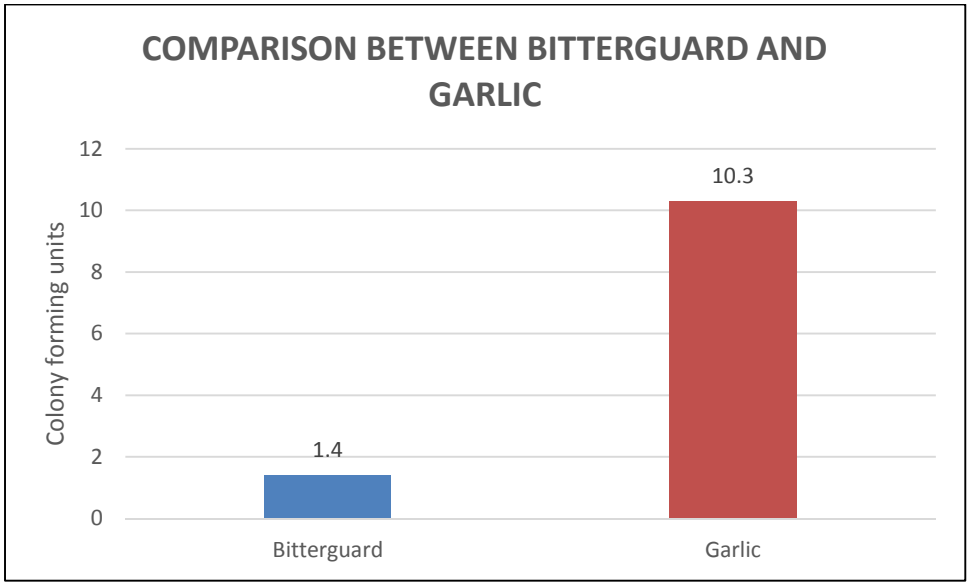
From **Table 3** following results were analyzed

- There was a significant difference between normal saline and Garlic, Bitter guard, NaOCl, CHX.
- There was a significant difference between NaOCl and Garlic, Normal saline & no significance difference between NaOCl and Bitter Guard, CHX.
- There was a significant difference between CHX and Garlic, Normal saline & no significance difference between CHX and Bitter Guard, NaOCl.
- There was a significant difference between Bitter Guard and Garlic, Normal saline & no significance difference between Bitter Guard and NaOCl, CHX.
- There was a significant difference between Garlic and Bitter guard, NaOCl, CHX, normal saline.

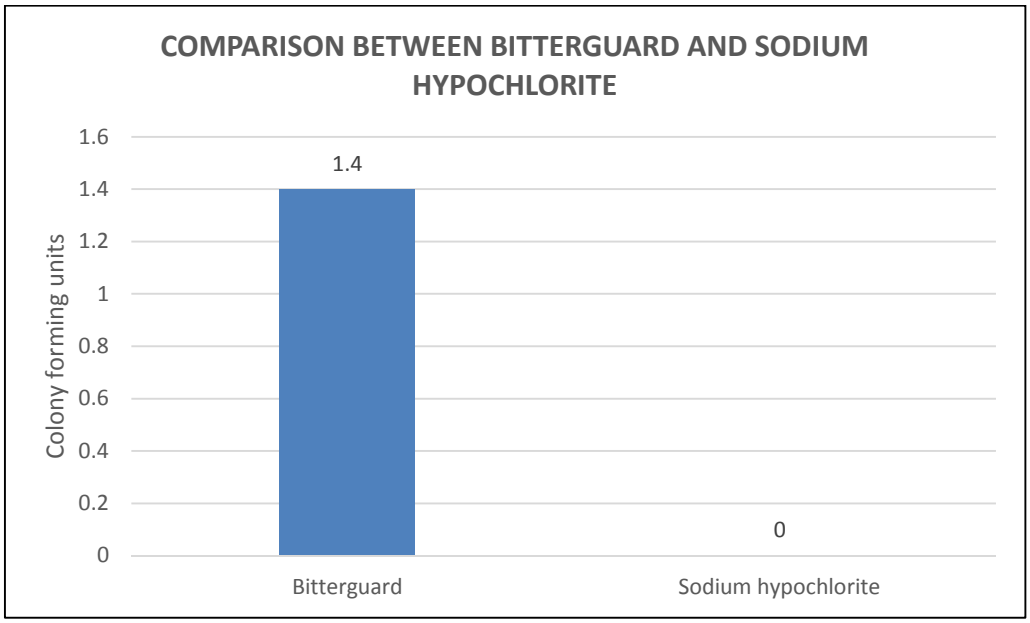
Graph 1



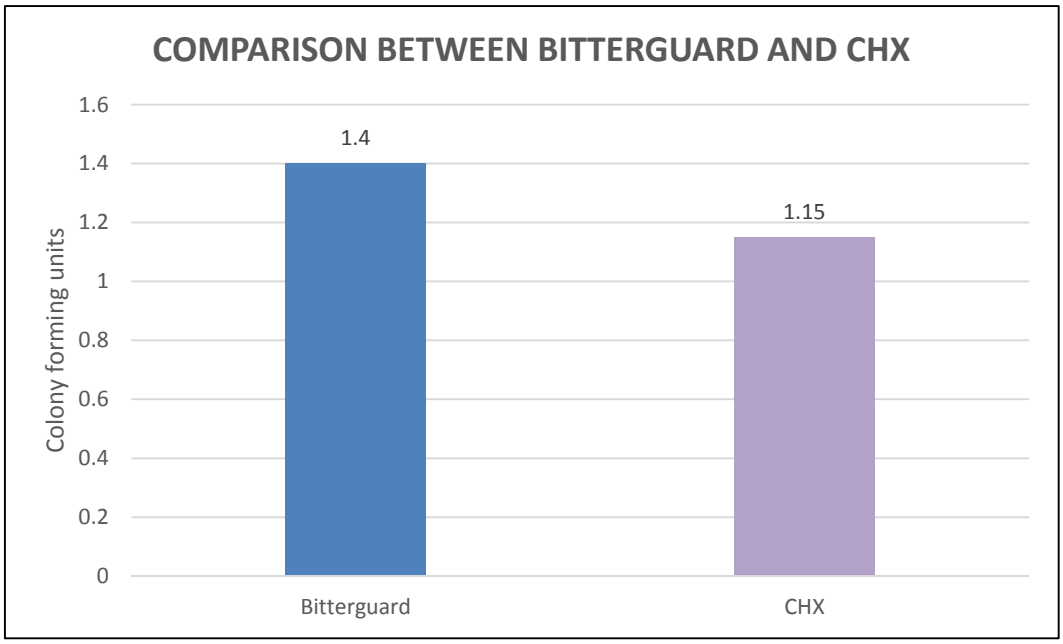
Graph 2:



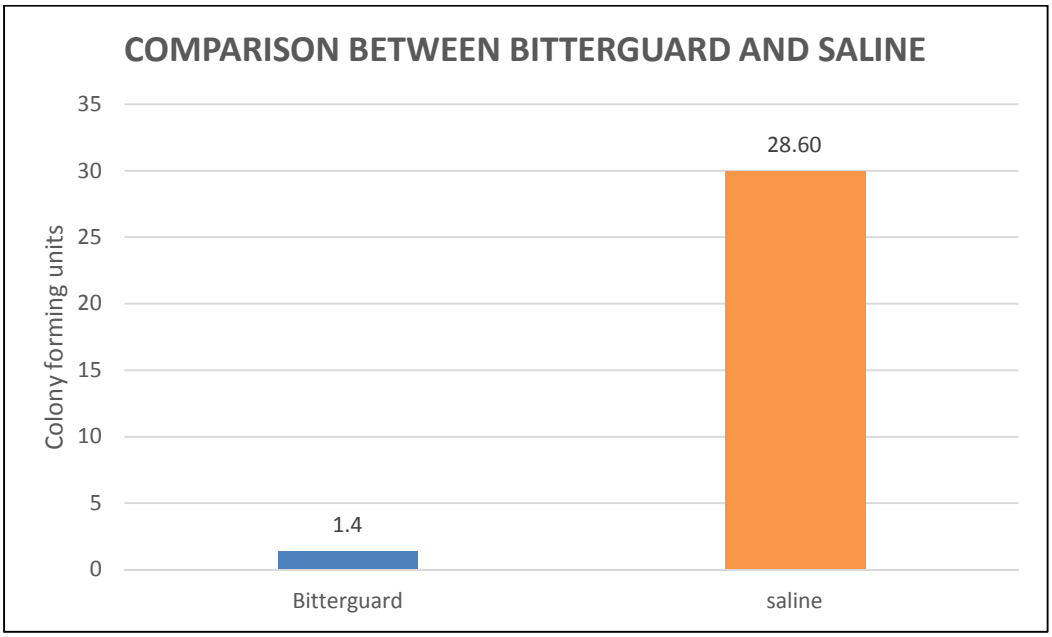
Graph 3:



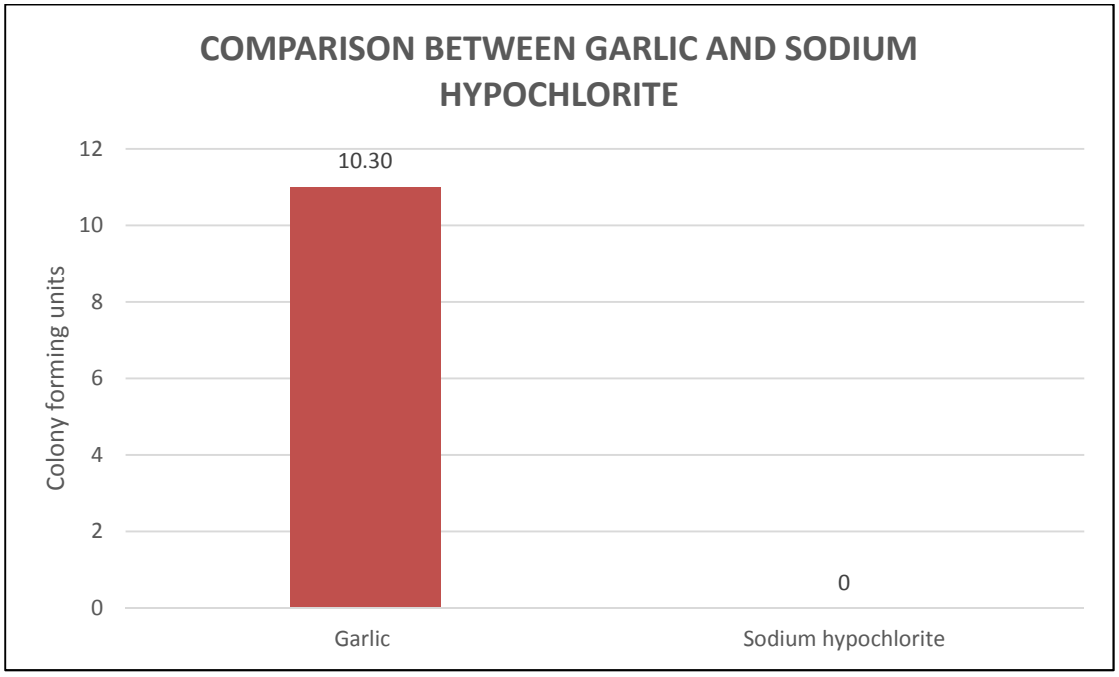
Graph 4:



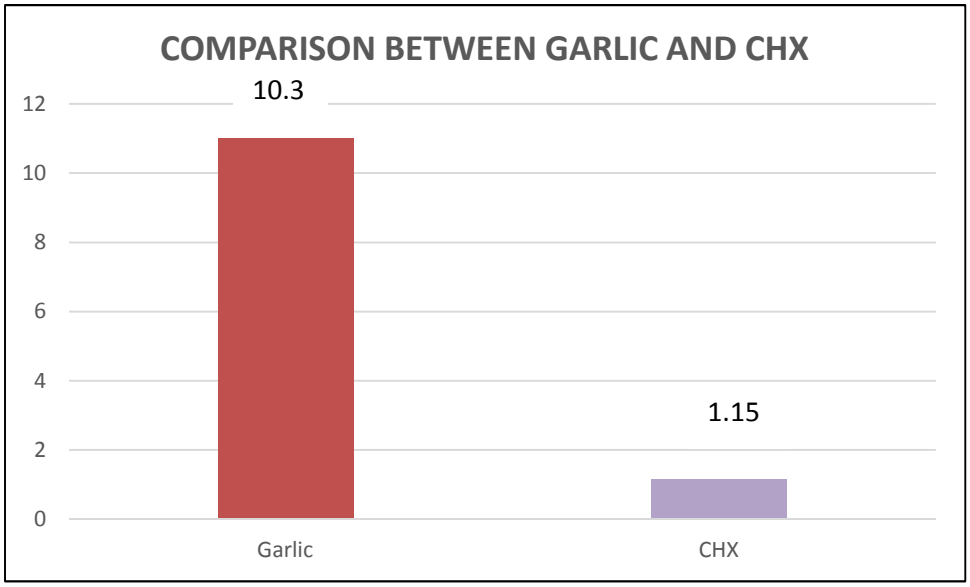
Graph 5:



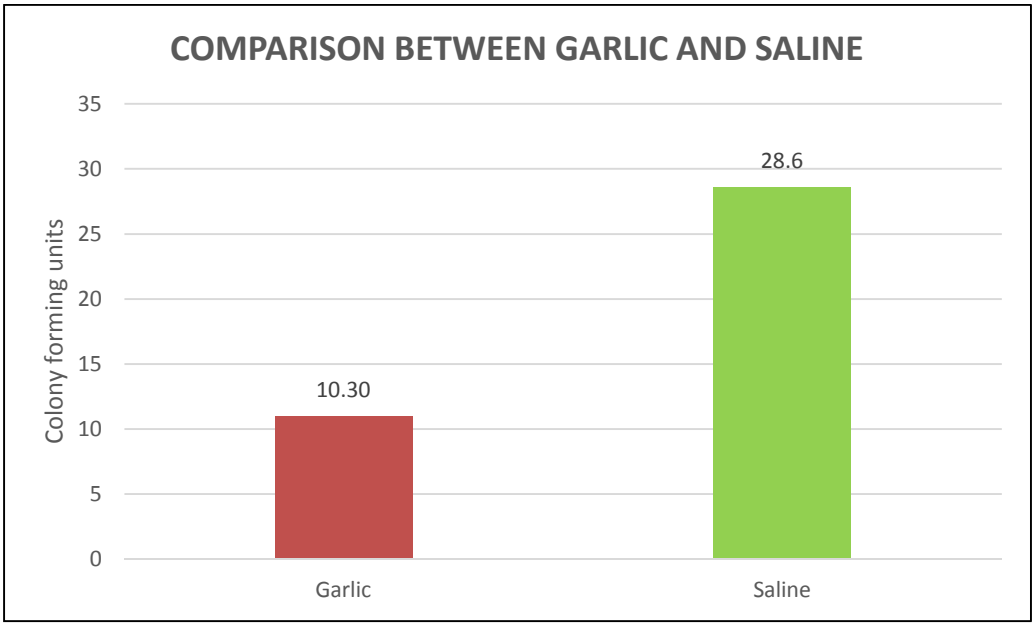
Graph 6:



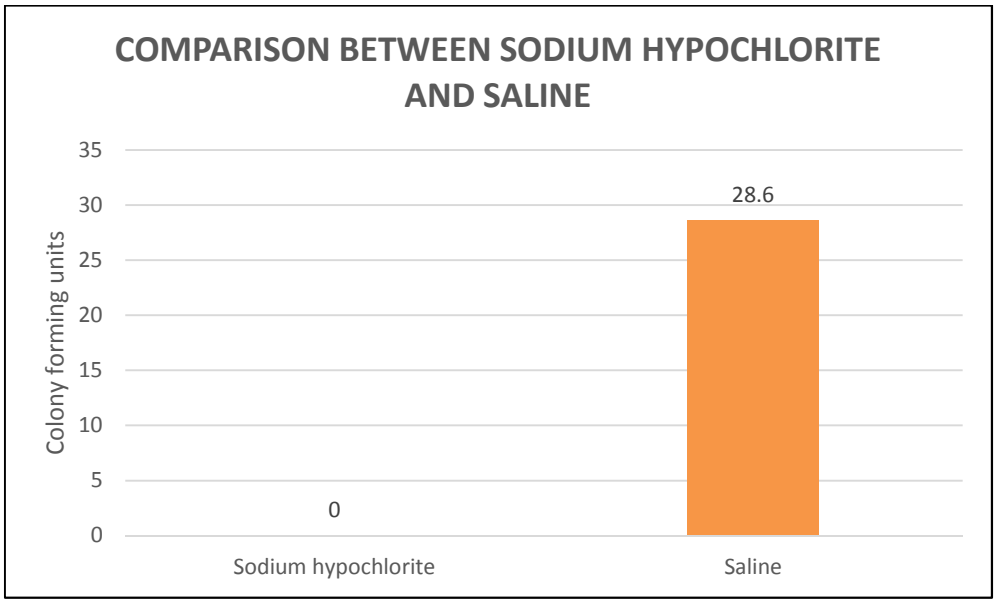
Graph 7:



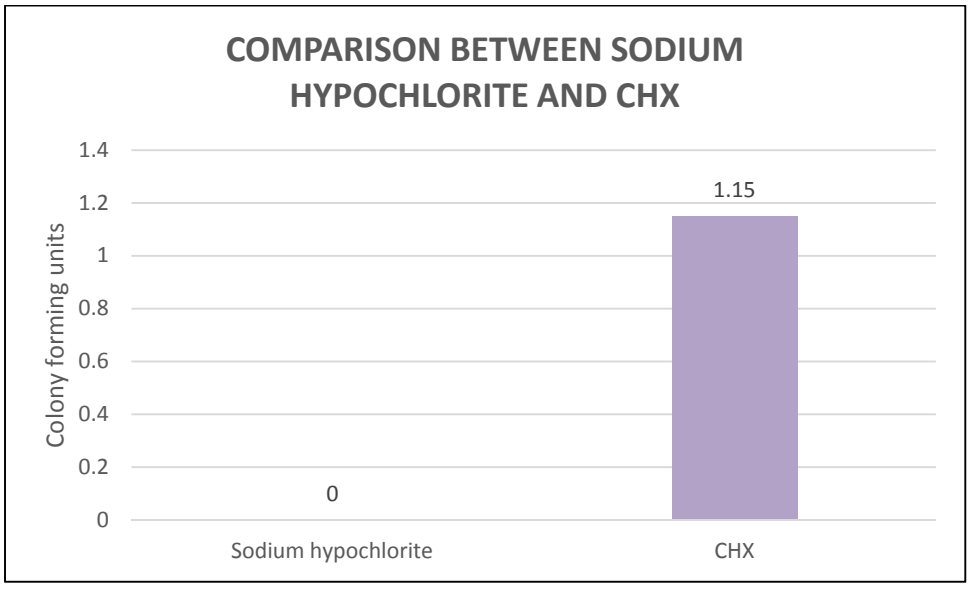
Graph 8:



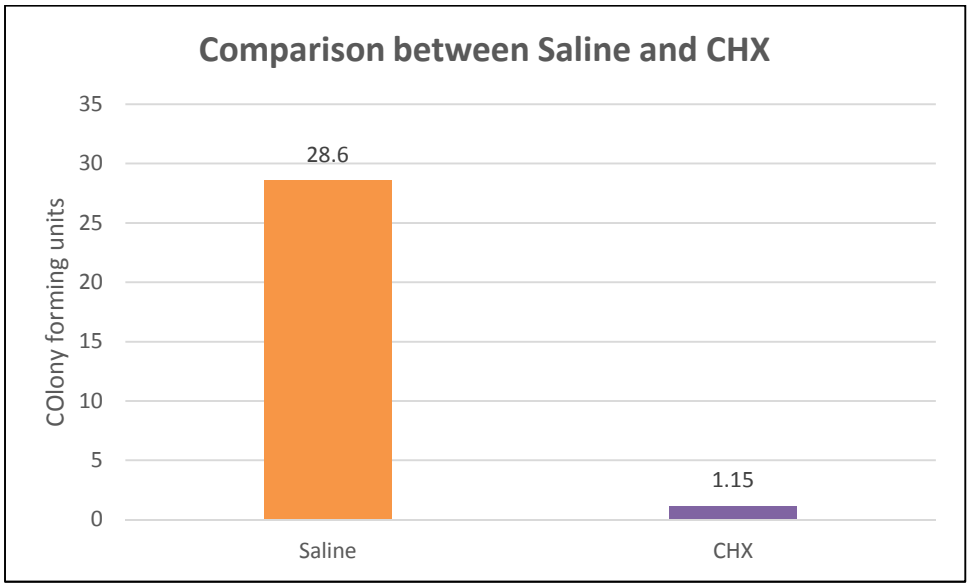
Graph 9:



Graph 10:



Graph 11:


Comparison between Saline and CHX
COlony forming units
35
30
25
20
15
10
5
0
28.6
1.15
Saline
CHX

DISCUSSION

Anton van Leuwenhoek was the first to observe oral flora. His description of ‘animalcules’ Observed with his microscopes included those from dental plaque and from an exposed pulp Cavity.[50]

In 1894 W. D. Miller was the first who published observations from the root canal with infected pulp space. Since that time bacteria was implicated in infections of endodontic origin. Further studies and development of anaerobic sampling techniques, demonstrated that the endodontic environment is selective and supports the growth of specific microorganisms.

Precise identification of microorganisms participating in the pathogenesis of apical periodontitis is important in order to understand the disease process and to provide effective antimicrobial treatment.[51] Bacteria have several possible pathways to invade the pulp. These include caries, enamel and dentine cracks, fractures, exposed and patent dentine tubules in the crown area or in the gingival/periodontal pocket, lateral canals, leaking restoration and a haematogenous pathway associated with bacteremia.[52]

The resident microbial flora in the oral cavity typically contains 10^{10} bacteria. However, only 150 microbial species have been isolated and cultured from root canals.

The endodontium is a sterile cavity and the invasion of oral microbes to establish infection is by the penetration of enamel and dentine and overwhelms the host responses.

Although all the bacteria in the oral cavity can invade the root canal, only a few microbes have been identified in infected root canals. Endodontic infections with E. faecalis are probably not derived from patients own micro flora, which indicates that in these infection E. faccalis is of exogenous origin.[53]

Enterococcus faecalis is the most commonly implicated microorganism in asymptomatic persistent infections. The highly complex nature of the organism poses a great Challenge for endodontists. Enterococci are gram positive cocci that can occur singly, in pairs or as short chains. They are facultative anaerobes, possessing the ability to grow in the presence or absence of oxygen. Enterococcus species live in vast quantities (10^5-10^8 CFU per gram of feces) in the human intestinal lumen and under most circumstances cause no harm to their hosts.[54]

Enterococci can withstand harsh environmental conditions. Enterococci can grow at 10° C and 45°C at pH 9.6 in 6.5% NaOC1 broth and survive at 60°C for 30 minutes. The ability of E faecalis to tolerate or adapt to harsh environmental conditions may act as an advantage over other species. It may explain its survival in root canal

infections, where nutrients are scarce and there are limited means of escape from root canal medicaments.[55, 56, 57]

The virulence factor E. faecalis includes lytic enzymes cytolysin, aggregation substance, pheromones and lipoteichoic acid. It has been shown to adhere to host cells, express proteins that allow it to compete with other bacterial cells and alter host responses. E. faecalis is able to suppress the action of lymphocytes, potentially contributing to endodontic failure.[58]

In the present E. faccalis was chosen as the test organism as it is the most commonly isolated intracanal bacteria from treatment failure cases, its association with persistent apical inflammation and its resistance to elimination by irrigating solutions and medicaments.[59] It has been used extensively in endodontic research because it has been detected in 63% of post treatment diseases[60] and due to the high level of resistance to a wide range of antimicrobial agents. MTCC 2527 strains of E.faecalis which has been the standard strain used in the previous studies was selected for the present study. In this study the cementum was left intact to simulate clinical conditions.[61] The minimum instrumentation size required for the penetration of irrigants in the apical third is 30.[62] The canals were enlarged till F3 Protaper universal rotary file in the present study.

E. faecalis can penetrate dentinal tubules to a depth of 300-400 μm within 3 weeks. Prolonged incubation period increased the number of infected dentinal tubules but depth of penetration of bacteria increases slowly with time.[63] Hence in this study the teeth were inoculated with the organism and incubated for 21 days.

In growth or progress of bacteria into the dentinal tubules could be delayed or prevented by the presence of a smear layer. Etching of dentin before exposure results in deeper penetration.[64] 17% EDTA was used in this study for removing the smear layer in the experimental specimens before autoclaving and inoculation. Another important factor for the survival of bacteria is the availability of a nutrient source.[65] The teeth were immersed in the streptococcus selection broth and the broth was replaced on alternate days during the 21 day incubation period. Subsequent change of the broth allowed the microorganism to rearrange in bio-films which is a structure known to confer resistance of microbial cells to different antimicrobial agents.[66]

Another reason for the replacement of the broth was to avoid medium saturation.[67] Sample preparation was done using 40 size H files which was similar tooth sample collection done in a previous study in 2007.[68] The methodology adopted for enumeration of CFU (Pour plate method) was in resemblance with the study conducted by Lynn et al.[69]

Chemomechanical preparation is a short term procedure and NaOCl remains in the canal for only a few minutes. So the antimicrobial effectiveness of NaOCl within the root canal depends on concentration and contact time.[70] In the present study a contact time of 5 minutes was taken as the standard time for all the irrigants. This could be explained on the basis of maximum antibacterial action exhibited by Cinnamon and Garlic in a 5 minute contact time during the pilot study. Results from previous studies have shown that 5.25% NaOCl can eliminate E. faecalis in a short exposure time of less than 30 seconds[1], 30 seconds[71], and 2 minute[72] which contradicts the findings of the present study. The difference in contact time may be attributed to the following factors.

- In the previous study there is direct contact of microorganism with the antimicrobial agent, bacterial suspension were mixed with the antimicrobial agent where as in the present study the dentinal tubules are inoculated with the organism to simulate the clinical condition.
- The inhibitory effect of dentin on the bactericidal effect of the irrigant has been documented.[73]

An important limitation of many studies when evaluating the endodontic microbia refers to sample preparation. In comparison to the study conducted by Berber et al 5.25% NaOCl eliminated strains of E.faecalis in a 10 minute contact time.[74] The methodology adopted in the latter study is similar to the present study except for the sample

preparation. Burs of different sizes were used to procure dentinal shavings unlike H files used in the present study. Bacterial inhibition at greater depths was assessed using burs of different sizes whereas H files were indicative of bacterial inhibition to a limited depth.

Chlorexidine has been used as an antibacterial agent in dentistry since 1962. It is cationic bis-biguanide, which is active against gram positive and gram negative bacterial spores, lipophillic virus, yeast and dermatophytes, being bacteriostatic at low concentrations and bactericidal at high concentrations. Several advantages for the clinical use of CHX as a root canal disinfectant include, its low toxicity, substantivity, more tolerable odor than sodium hypochlorite, better taste and non bleaching effects.[75]

For endodontic purposes, generally chlorhexidine can be used in a liquid preparations. Ferraz et al. showed that 2% CHX has several advantages of antimicrobial activity, substantivity and biocompatibility properties.[72] But the most important disadvantage of CHX is its inability to dissolve remnants of necrotic tissues and chemically clean the root canal system.[76]

Chlorhexidine has been used in endodontics and proposed both as an irrigant and an intracanal medicament. When used as an intracanal medicament, Chlorhexidine is more effective than Calcium Hydroxide against E. faccalis infection in dentinal tubules. Chlorhexidine has also

been shown to have long-term antimicrobial properties because of its unique ability to bind to hydroxyapatite. A gradual release of this bound chlorhexidine could maintain an even level of the molecule sufficient to create a bacteriostatic effect in the root canal over a prolonged period of time. This is in contrast to the effect of other disinfectants, which rapidly dissipate from the pulp space and have no residual antimicrobial effects.[76]

To overcome problems associated with currently used irrigants, use of natural plant extracts as endodontic irrigants might be of interest to professionals as part of a growing trend to seek natural remedies in dental treatment. The role of natural extracts for endodontic purpose has been evaluated for plants such as Arctium lappa Morinda citrifolia Triphala. Green Tea Polyphenols and Liquorice, Allium Sativum. Garlic, Cinnamomum zeylanicum, Azadiracta indica, Ginger extract, Mishwak, Aloe vera Linne Myristica fragans, Teminalia chebula, in terms of their antimicrobial efficacy against E. faecalis.[3, 25,26,27,43,77]

There are only very few limited studies on Bitter Guard (Momordica charantiao) and Garlic (Allium sativum) as endodontic irrigants against E. faecalis. Hence in the present study these two herbal extracts are tested with the commonly used 5.25% Naocl and 2% CHX endodontic irrigants against E. faecalis.

Garlic (Allium Sativum) is a bulbous perennial medicinal plant which belongs to the family Liliaceae. The antmicrobial activity is attributed to thiosulphinates. Garlic can be used on microorganism that has particularly developed resistance to antibiotics. Multidrug resistant E.faecalis can be made vulnerable by the medicinal properties of garlic. Studies proved that extracts of garlic are bactericidal and are effective against E.coli, S.aureus B.cereus, Salmonella, listeria, proteus and Streptococcal species.[36]

Bitter Guard (Momordica charantia) a member of the Cucurbitacea family, has long been used as food and medicine. Antioxidant, anti diabetes, anti inflammatory, anti bacterial and anti cancer effectrs of M. charantia have been reported (Grover and Yadav, 2004; Budrat and Shotipruk, 2009). Fruits and seeds of M. charantia possess medicinal properties such as anti-HIV, anti-ulcer, anti-inflammatory, anti-leukemic, antimicrobial and antitumor (Taylor, 2002). The plant was generally used to investigate for immunostimulant activity, chemotaxis stimulation, treating ulcers, antihyperglycemic and hypoglycemic activity and antioxidant enzyme activities. In addition, it was reported to exhibit diverse biological activities such as being antimicrobial.[78]

Sodium hypochlorite in present study show complete inhibition of E. faecalis with mean CFU of 0.00. These results are comparable to the previous studies where NaOCl shows 100% inhibition of growth of

E. faecalis. the biomechanical preparation action on microorganisms and endotoxins by using sodium hypochlorite and an intracanal medication containing Zingiber officinale, with or without calcium hydroxide. The results showed that the NaOCl eliminated 100% of root canal microorganisms and reduced 88.8% of endotoxins immediately after biomechanical preparation.[28] compare the antimicrobial activity of extracts of Aloe vera, garlic, and 5% NaOCl against E. faecalis. They concluded that NaOCl, was considered as gold standard, also showed higher zones of inhibition.[32]

Other commonly used endodontic irrigant was 2% chlorhexidine. In the present study 2% CHX showed mean CFU of (1.14×10^{-3}) and Bitter guard showed mean CFU of (1.40×10^{-3}). Hence bitter guard is comparable to 2% CHX. Chandrappa PM et al 2015 assessed the antimicrobial activity of herbal medicines tulasi extract and neem extract and chlorhexidine against E. Faecalis and they concluded that both herbal extracts showed significant inhibitory effect against E. Faecalis comparable to 2% chlorhexidine.[35] Chlorhexidine is known to be more effective antimicrobial agent as compared with NaOCl, substantively low toxicity and no tissue dissolving properties (Soares et al 2008).[79]

In the present study garlic showed mean CFU of (10.30×10^{-3}) which is comparatively higher than bitter guard, 2% CHX and 5.25% NaOCl. Eswar et al showed 2% CHX showed better antimicrobial

efficacy compared to garlic extract, which is concurrence with the present study, as in this study 2% CHX showed better antibacterial efficacy compared to bitter guard and garlic extracts. The possible reasons might be due to bactericidal dosage of 2% CHX and increased diffusion of the medicament into the dentinal tubules. Chlorhexidine is a positively charged hydrophobic and lipophilic molecule that interact with phospholipids and lipopolysacharides on the cell membrane of bacteria and enter the cell through some type of active or passive transport mechanism. As a consequence, the cytoplasm becomes congealed, with resultant reduction in leakage; thus, there is a biphasic effect on membrane permeability.[80]

The means CFU from low to high with all irrigants tested was as follows Group 2 - 5. 25% NaOCl (0.00); Group 3- 2% Chlorhexidine (1.14×10^{-3}); Group 4- Bitter Guard (1.40×10^{-3}); Group 5- Garlic (10.30×10^{-3}) and Group 1- Normal saline (28.60×10^{-3}).

The inter group comparison between normal saline and Garlic, Bitter guard, 5.25% NaOCl, 2% CHX there was a significance difference (p=0.000).

The inter group comparison between 5.25% NaOCl and Garlic and Normal saline there was a significance difference (p=0.000) & there was no significance difference between 5.25% NaOCl and Bitter Guard (p= 0.646); 5.25% NaOCl and 2% CHX (p=0.790).

The inter group comparison between 2% CHX and Garlic, Normal saline there was significance difference (p=0.000) & there was no significance difference between 2% CHX and 5.25% NaOCl (p=0.790); 2% CHX and Bitter guard (p=0.999).

The inter group comparison between Bitter Guard and Garlic, Normal saline there was significance difference (p=0.000) & there was no significance difference between Bitter guard and 5.25% NaOCl (p= 0.646); Bitter guard and 2% CHX (p=0.999).

Inter group comparison between Garlic and Bitter guard, 5.25% NaOCl, 2% CHX, normal saline there was a significance difference (p=0.000).

Herbal extracts Bitter Guard (Momordica charantiao) and Garlic (Allium sativum) are effective against E. faecalis. Among the two Bitter Guard (Momordica charantiao) was more effective than Garlic (Allium sativum), which is nearly equal to 2% Chlorhexidine.

Generally plant extracts are usually more active against gram positive bacteria than gram negative (Basri and Fan, 2005). This may be due to the permeability barrier provided by the cell wall or the membrane accumulator mechanisms.[15] CFU were used in the present

study beacuase they allow quantification of cultivable bacteria per millgrams of dentine.[81]

Since time immemorial, 5.25% NaOCl has been regarded as the most effective endodontic irrigant, not only because it has a high antimicrobial effect, but also because of its tissue dissolving action which has not bee foud in any other endodontic irrigant. But its usage at high concentrations is undesirable as it is an irritant to the periapical tissues.[82]

Contrary to NaOCl other commonly used alternate endodontic irrigant was Chlorhexidine. The constant increase in antimicrobial resistance and side effects caused by synthetic drugs has prompted researchers for alternatives. In recent years there is an exponential growth in the field of herbal medicine because of their natural origin, easy availability, efficacy, safety and less side effects.

In the light of the problems associated in usage of high Naocl concentrations and promising results obtained in the present study with Bitter Guard (Momordica charantia) and Garlic (Allium sativum) assures a long and successful use in endodontic field. Bitter Guard is equally effective as compared to Chlorhexidine.

Further clinical and in vitro studies determining its tissue dissolving efficacy and establishing this herbal extracts usage as endodontic irrigant is the need of the hour.

SUMMARY

During endodontic treatment, number of microorganisms within the root canals is reduced as much as possible using mechanical and chemical procedure. However, there is a possibility that some of them are left in the canal. That is why various medications are placed inside the canals during the time period between treatment sessions. Persistent endodontic infections are mainly due to retention of microorganism in the dentinal tubules. Enterococcus faecalis is the primary organism detected in persistent asymptomatic infections. Due to the disadvantages of these irrigants like toxicity and synthetic concern, consumption of preparations from medicinal plants has increased over the last few decades. The aim of current study was to evaluate the efficacy of two herbal extracts i.e, Bitter Guard (Momordica charantia) and Garlic (Allium sativum) as endodontic irrigants against Enterococcus faecalis.

Fifty Single rooted human mandibular premolar extractes for orthodontic reasons. Teeth were decoronated to standardize the length to 12-15mm. Cleaning and shaping of root canals were done by crown down technique using protaper universal rotary files till F3. Specimens were placed in steel containers containing BHI broth and sterilized in autoclave. From a stock culture of MTCC 2527 E.faecalis strain, subculture was made onto a plate of Diagnostic Sensitivity Test Agar. Enumeration of live bacteria (CFU) was carried by serial dilution method. For injecting into the tooth a suspension of bacteria containing

10u CFU per ml was used. The root canals were inoculated with E.Faecalis suspension and incubated at 37° c for 21 days. The specimens were divided into five groups, each containing ten teeth. Test irrigating solutions were used as follows. Group 1 - Normal Saline, Group 2 - 5.25% NaOCl, Group 3 - 2% CHX, Group 4 – Bitter guard, Group 5 – Garlic. Dentinal shavings were collected using no 40 H file in an aseptic condition. Shavings were transferred into test tubes containing 10 ml sterile normal saline ($10^{-1)}$. Three serial dilution was carried out i.e., till 10^{-3}. From this 1 ml was pipetted on to a sterile 100 mm diameter disc & to these plates 15 ml of melted agar medium was added and allowed to solidify. Plates were incubated for 24hours at 37° C. After incubation the number of colonies were counted.

The results of the present study shows that the means CFU from low to high with all irrigants tested was as follows Group 2 - 5. 25% NaOCl (0.00); Group 3- 2% chlorhexidine (1.14×10^{-3}); Group 4- Bitter Guard (1.40×10^{-3}); Group 5- Garlic (10.30×10^{-3}) and Group 1- Normal saline (28.60×10^{-3}).

Promising results obtained in the present study with Bitter Guard (Momordica charantia) and Garlic (Allium sativum) assures a long and successful use in endodontic field. Bitter Guard is equally effective as compared to Chlorhexidine.

CONCLUSION

With in the limitations of the present in vitro study, based on the employed methodology and according to the results obtained, it was concluded that

1. 5.25% NaOCl showed complete inhibition of E. faecalis and proved as a gold standard endodontic irrigant.
2. Bitter Guard (Momordica charantia) and 2% Chlorhexidine are equally effective against E. faecalis.
3. Inhibition of E. faecalis was more effective with Bitter Guard (Momordica charantia) compared with Garlic (Allium sativum).
4. Herbal extracts Bitter Guard (Momordica charantia) and Garlic (Allium sativum) are effective against E. faecalis.
5. Normal saline has least effective against E. faecalis of all the tested irrigants.

REFERENCES

1. Gomes B P F A, Souza F C S, Feraz C C R et al. Effectiveness of 2% Chlorhexidine gel and calcium hydroxide against Entero coccus faecalis in bovine root dentine in vitro. Int Endod J, 2003;36:267-75.
2. Ahangari Z, Eslami G, Ghannad S. Antimicrobial activity of propalis in comparision with calcium hydroxide against Entero coccus faecalis – An in vitro study. J Dental School, 2012; 30(1):9-7.
3. Santhini gopalakrishnan, Rajesh S, Jotish ravi. A comparitive evaluation of antimicrobial efficacy of cinnamon and garlic as endodontic irrigants against enterococcus faecalis - an in vitro study. Endodontology, 2014;26(1):149-157.
4. Ravi Shankar P, Lakshmi T, Aravind Kumar S. Ethno – Botanical approach for root canal treatment, an update. J. Pharm. Sci. & Res.,2011;3(10):1511-19.
5. Pujar M, Makandar S. Herbal uasage in Endodontics – A review. Int. J Contemporary Dentistry. 2011;2(1):34-7.
6. Sushma Jaju, Prashant P.Jaju. Newer Root Canal Irrigants In Horizon: A Review. International Journal of Dentistry. 2011:1-9
7. Madhu Pujar, ChetanPatil, AjayKadam. Comparison of antimicrobial efficacy of Triphala, Green tea polyphenols and 3% Sodiumhypochlorite on Enterococcus faecalisbiofilims formed on tooth substrate – In vitro. JIOH 2011;3(2):23-29.

8. Matthias Zehnder. Root Canal Irrigants. J Endod 2006;32(5):389 -98.
9. Hossain M M, Paul N, Sohrab M H, Rahman E, Rashid M A. Antimicrobial activity of Vitex trifolia. Fitoterapia, 2001;72:695-97.
10. Arora T, Kang RS, Mann JS, Khurana NS, Aggarwal R, Walia G. Antimicrobial activity of herbal extracts against recalcitrant endodontic pathogens: An original in vitro study. Saint Int Dent J. 2015;1:28-32.
11. Srinivasan Durairaj, Sangeetha Srinivasan, P.Lakshmanaperumalsamy. Invitro antibacterial activity and stability of Garlic extract at different PH and temperature. Electronic Journal of Biology. 2009;5(1):5-10.
12. Dhinahar. S, Lakshmi. T. Role of botanicals as antimicrobial agent in management of dental infections – A Review. International Journal of Pharma And Biosciences. 2011;2(4):690-704.
13. Enzo A. Palombo. Traditional medicinal plant extract and natural products with activity against oral bacteria. Potential application in the prevention and treatment of oral Diseases. Evidence Based Complementary and Alternative Medicine, 2011:1-12.
14. Dhanyakumar, N M PreenaSidhu. The antimicrobial activity of AzadirachtaIndica, Glycyrrhizaglabra, Cinnamum zeylanicum, Syngium Aromaticum, Accacia Nilotica on streptococcus Mutans

and Enterococccus faecalis- An in vitro study.Endodontology:18-25.

15. Hernandez M. M, Heraso C, Villarreal M. L, Vagas – Arispuro I, Aranda E. Biological activities of crude plant extract from Vitex trifolia (Verbenaceae). J Ethno Pharmacology. 1999;67:37-44.
16. Nageshwar Rao R, Kidiyoor h K and Hedge c. Efficacy of calcium hydroxide – chlorhexidine paste against Enterococcus faecalis, an in vitro study. Endodontology 2004;16:61-64.
17. Valera MC, Salvia AC, Maekawa LE, Camargo SE, Carvalho CA, Camargo CH, Koga-Ito CY. Antimicrobial analysis of chlorhexidine gel and intracanal medicaments against microorganisms inoculated in root canals. Minerva Stomatol. 2010 Jul-Aug;59(7-8):415-21.
18. Kannathasan K, Senthilkumar A, Venkatesalu V. Antibacterial potential of some Vitex species against human pathogenic bacteria an in –vitro study. Asian pacific J tropical medicine. 2011;645-8.
19. Ehsani M, Amin Marashi M, Zabihi E, Issazadeh M, Khafri S. A Comparison between Antibacterial Activity of Propolis and Aloe vera on Enterococcus faecalis (an In Vitro Study). Int J Mol Cell Med. 2013;2(3):110-6.
20. Valera MC, Maekawa LE, de Oliveira LD, Jorge AO, E, Carvalho CA. In vitro antimicrobial activity of auxiliary chemical substances and natural extracts on Candida albicans and

Enterococcus faecalis in root canals. J Appl Oral Sci. 2013;21(2):118-23.

21. Maekawa LE, Valera MC, Oliveira LD, Carvalho CA, Camargo CH, Jorge AO. Effect of Zingiber officinale and propolis on microorganisms and endotoxins in root canals. J Appl Oral Sci. 2013;21(1):25-31.
22. Ghonmode WN, Balsaraf OD, Tambe VH, Saujanya KP, Patil AK, Kakde DD. Comparison of the antibacterial efficiency of neem leaf extracts, grape seed extracts and 3% sodium hypochlorite against E. feacalis - An in vitro study. J Int Oral Health. 2013;5(6):61-6.
23. Kumar H. An in vitro evaluation of the antimicrobial efficacy of Curcuma longa, Tachyspermum ammi, chlorhexidine gluconate, and calcium hydroxide on Enterococcus faecalis. J Conserv Dent. 2013;16(2):144-7.
24. Castilho AL, Saraceni CH, Díaz IE, Paciencia ML, Suffredini IB. New trends in dentistry: plant extracts against Enterococcus faecalis. The efficacy compared to chlorhexidine. Braz Oral Res. 2013;27(2):109-15.
25. Hannah Rosaline, Kandaswamy D, Gogulnath D, Rubin MI. Influnece of various herbal irrigants as a final rinse on the adherence of enterococcus faecalis by fluorescence confocal laser scanning microscope. J Conserv Dent. 2013;16(4):352-5.
26. A. Gupta, J. Duhan, S. Tewari, P. sangwan, A. Yadav, G. Singh, R. Juneja & H. Saini. Comparative evaluation of antimicrobial

efficacy of Szzygium aromaticum, Ocimum sanctum and Cinnamomum Zeylanicum plant extract against Enterococcus faecalis: a preliminary study. Int Endod J. 2013;46:775-83.

27. Thilla S Vinothkumar, Mohammed I Rubin, Lakshmi Balaji, Deivanayagam Kandaswamy. In vitro evaluation of five different herbal extracts as ann antimicrobial endodontic irrigant using real time quantitative polymerase chain reaction. J Conserv Dent.2013;16(2):167-70.
28. Valera MC, Maekawa LE, Chung A, Cardoso FG, Oliveira LD, Oliveira CL, Carvalho CA. The effect of sodium hypochlorite and ginger extract on microorganisms and endotoxins in endodontic treatment of infected root canals. Gen Dent. 2014;62(3):25-9.
29. Mistry KS, Sanghvi Z, Parmar G, Shah S. The antimicrobial activity of Azadirachta indica, Mimusops elengi, Tinospora cardifolia, Ocimum sanctum and 2% chlorhexidine gluconate on common endodontic pathogens: An in vitro study. Eur J Dent. 2014;8(2):172-7.
30. Sponchiado EC, Pereira JV, Marques AA, Garcia Lda F[1], França SC. . In vitro assessment of antimicrobial activity of Pothomorphe umbellata extracts against Enterococcus faecalis. Indian J Dent Res. 2014;25(1):64-8.
31. Birring OJ, Viloria IL, Nunez P. Anti-microbial efficacy of Allium sativum extract against Enterococcus faecalis biofilm and

its penetration into the root dentin: An in vitro study. Indian J Dent Res. 2015;26(5):477-82.

32. Karkare SR, Ahire NP[1], Khedkar SU. Comparative evaluation of antimicrobial activity of hydroalcoholic extract of Aloe vera, garlic, and 5% sodium hypochlorite as root canal irrigants against Enterococcus faecalis: An in vitro study. J Indian Soc Pedod Prev Dent. 2015;33(4):274-8.
33. Mathew J, Pathrose S, Kottoor J, Karaththodiyil R, Alani M, Mathew J . Evaluation of an Indigenously Prepared Herbal Extract (EndoPam) as an Antimicrobial Endodontic Irrigant: An Ex Vivo Study. J Int Oral Health. 2015;7(6):88-91.
34. Saxena D, Saha SG, Saha MK, Dubey S, Khatri M. An in vitro evaluation of antimicrobial activity of five herbal extracts and comparison of their activity with 2.5% sodium hypochlorite against Enterococcus faecalis. Indian J Dent Res. 2015 Sep-Oct;26(5):524-7.
35. Chandrappa PM, Dupper A. Antimicrobial activity of herbal medicines (tulasi extract, neem extract) and Chlorhexidine against Enterococcus Faecalis in Endodontics: An in – vitro study. J Int Soc Prev Community Dent. 2015;5(2):89-92.
36. I. N. Radwan, B. Randa, A. N. Hend, G. Camilia. Evaluation of antimicrobial efficacy of four medicinal plant extracs used as root canal irrigant ofn Enterococcus faecalis: An in vitro study. International Dental & Medical Journal of Advanced Research. 2015;1:1-8.

37. Zeenath Ambareen, Sapna Konde, Sunil Raj N, Kumar N C. Antimicrobial efficacy of herbal extracts.Int J Oral Health Dentistry. 2015;1(3)108-113.

38. Mustafa M. Antibacterial Efficacy of Neem (Azadirachta indica) Extract against Enterococcus faecalis: An in vitro Study. J Contemp Dent Pract. 2016;17(10):791-4.

39. Valera MC, Oliveira SA, Maekawa LE, Cardoso FG, Chung A, Silva SF, Carvalho CA. Action of Chlorhexidine, Zingiber officinale, and Calcium Hydroxide on Candida albicans, Enterococcus faecalis, Escherichia coli, and Endotoxin in the Root Canals. J Contemp Dent Pract. 2016 Feb 1;17(2):114-8.

40. Mathew J, George R, Theruvil R, Padavil TC, Tomy L, Kurian A. Antibacterial Activity of Leaf Extract of Annona muricata and Simarouba glauca on Enterococcus faecalis. J Contemp Dent Pract. 2016 Aug 1;17(8):650-3.

41. Shenoi PR, Morey ES, Makade CS, Gunwal MK, Khode RT, Wanmali SS. In vitro evaluation of the antimicrobial efficacy of chitosan and other endodontic irrigants against Enterococcus faecalis. Gen Dent. 2016 Sep-Oct;64(5):60-3.

42. Jose J, Krishnamma S, Peedikayil F, Aman S, Tomy N, Mariodan JP. Comparative Evaluation of Antimicrobial Activity of QMiX, 2.5% Sodium Hypochlorite, 2% Chlorhexidine, Guava Leaf Extract and Aloevera Extract Against Enterococcus faecalis and Candida albicans - An in-vitro Study. J Clin Diagn Res. 2016;10(5):ZC20-3

43. Babaji P, Jagtap K, Lau H, Bansal N, Thajuraj S, Sondhi P. Comparative evaluation of antimicrobial effect of herbal root canal irrigants(Morinda citrifolia, Azadirachta indica, Aloe vera) withsodium hypochlorite: An in vitro study. J Int Soc Prevent Communit Dent 2016;6:196-9.

44. Tonea A, Badea M, Oana L, Sava S, Vodnar D. Antibacterial and antifungal activity of endodontic intracanal medications. Clujul Med. 2017;90(3):344-347.

45. Nourzadeh M, Amini A, Fakoor F, Raoof M, Sharififar F. Comparative Antimicrobial Efficacy of Eucalyptus Galbie and Myrtus Communis L. Extracts, Chlorhexidine and Sodium Hypochlorite against Enterococcus Faecalis. Iran Endod J. 2017;12(2):205-210.

46. Yadav P, Chaudhary S, Saxena RK, Talwar S, Yadav S . Evaluation of Antimicrobial and Antifungal efficacy of Chitosan as endodontic irrigant against Enterococcus Faecalis and Candida Albicans Biofilm formed on tooth substrate. J Clin Exp Dent. 2017 Mar 1;9(3):e361-e367.

47. Ehsani M, Amin Marashi M, Zabihi E, Issazadeh M, Khafri S . A Comparison between Antibacterial Activity of Propolis and Aloe vera on Enterococcus faecalis (an In Vitro Study). Int J Mol Cell Med. 2013 Summer;2(3):110-6.

48. Sharma Neeraj, Pooni Neeraj, Singh Manmohan, Verma Kamal, Kaur Manjot, Dhiman Neha, Kamini and Bhandari Neeraj. A

review on karela. World journal of pharmacy and pharmaceutical sciences 2017; 6(4), 327-45.

49. Mohamed A. Eltawee. Antibacterial Effect of Garlic (Allium Sativum) On Staphyloccus Aureus: An In Vitro Study. Int'l Conf. on Advances in Environment, Agriculture & Medical Sciences 2014;16-17:47-9.
50. Teles A M, Manso M C, Lourerio S, Pina C and Cabeda J M. Microorganisms: The reason to perform endodontics. Formatex 2013;1778-86.
51. Peciuliene V, Maneliene R, Balcikonyte E. Microorganisms in root canal infections A Review. Stomatologi Baltic Dental and Maxillofacial Journal 2008;10(1):4-9
52. Haapasalo M, Udnaes T and Endal U. Persistant, recurrent and acquired infection of root canal system post treatment. Endodontic topics 2003;6:29-56.
53. Bhonchal Bharadwaj S B. Role of Enterococcus faecalis in failure of endodontic treatment- Review Article. Int J Curr. Microbial App. Sci 2013;2(8):272-7.
54. Stuart C H, Schwartz S A. Enterococcus faecalis: Its role in root canal treatment failure and current concepts in retreatment. JOE 2006;32(2):93-8.
55. Kayaoglu G and Orstavik D. Virulence factors of enterococcus faecalis: relation ship to endodontic disease. Crit Rev Oral Biol Med. International American Association for Dental Research 2004;15(5):308-20.

56. Radcliffe C , Potouridou L. Antimicrobial activity of varying concentrations of sodium hypochlorite on endodontic microorganisms Actimnomyces israelli, A. naeslundii, C. albicans and E. faecalis. Int End J 2004;37:438-46.
57. Ashraf f Fouad. Text book of Endodntic microbiology. 2009.
58. Suvarna R, Bhat S s and Hegde K S. Antibacterial activity of turmeric against Enterococcus faecalis- An in vitro study. Int. J. Curr. Microbial App. Sci. 2014;3(2):498-504.
59. Garlapati R, Venigalla B S. Comparison of the antimicrobial efficacy of two antibiotics Sparfloxacin and Augmentin as experimental root canal irrigating solutions against E. facecalis- An in vitro study. Journal of clinical and diagnostic Research 2016;10(3):ZC57-60.
60. Hancock HH, Sigurdsson A, Trope M, Moiseiwitsch J.Bacteria isolated after unsuccessful endodontic treatment in a North American population.Oral surgery, Oralmedicine, Oralpathology, Oral radiology and Endodontics 2001;91:579-86.
61. Russell. S. Eddy, Anthony P. Joyce, Steve Roberts, Thomas. B. Buxton, Frederick Liewehr. An In vitro evaluation of the antibacterial efficacy of Chlorine Dioxide on E. Faecalis in bovine Incisors – J Endod 2005;31(9):672-675.
62. Khademi A, Yazdizadeh M, Feizianfard M. Determination of the minimum instrumentation size for penetration of irrigants to the apical third of root canal systems. J Endod 2006;32:417-420.

63. Haapasalo M, Orstavik D.In vitro infection and disinfection of dentinal tubules. Journal of Dental Research 1987;66:1375-1379.

64. Safavi KE, Spanberg L, Langeland K.Smear layer removal effects on root canal dentin tubule disinfection.J Endod1989;15:175-176.

65. Adriaens PA, de Boever JA, Loesche WJ. Bacterial invasion in root cementum and radicular dentine of periodontally teeth in humans. Journal of Periodontology 1988;59;222-230.

66. Thomas R. Dunavant, John. D. Regan, Gerald N. Glickman, Eric S. Solomon, Allen L. Honeyman. Comparative evaluation of endodontic irrigants against Enterococusfaecalis Bio Filims. J Endod 2006;32 (6): 527 – 531.

67. Bettina Basrani, J.Miguel Santos, Leo Tjaderhane, Helen Grad, Omer Gorduysns, Junfu Huang, Herenia.P. Lawrence, Shimon Friedman. Substantive antimicrobial activity in Chlorhexidine-treated human root dentin. Oral Surg, Oral Med, Oral Pathol, Oral Radiol,Endod 2002;94: 240-245.

68. Eldeniz AU, Ozer F, Hadimli HH, Erganis O. Bactericidal activity of Er,Cr:YSGG laser irradiation against E.faecalis compared with NaOCl irrigation: an ex vivo piolot study. International Endodontic Journal2007;40(2):112-9.

69. Lynne RE, Lieweher FR, Patton WR, Buxton TB, McPherson JC.In Vitro Antimicrobial Activity of Various MedicationPreparations on E.faecalis in Root Canal Dentin .J Endod 2003;29:187-190.

70. Siqueira JF, Rocas IN, Faviera A, Lima KC. Chemomechanical reduction of the bacterial population in the root canal after instrumentation and irrigation with 1.0,2.5 and 5.25% NaOCl.J Endod2000;26:331-4.

71. N.T Sena, B.P.F.A Gomes, M.E Vianaa, V.B Berber, A.A Zaia,C.C. R Ferraz, F.J Souza–Filho. In vitro antimicrobial activity of sodium hypochlorite and chlorhexidine against selected single-species biofilim. International Endodontic Journal 2006;39(11):878-885.

72. C.E. Radcliffe, L. Potouridou, R. Qureshi, N. Habahbeh, A. Qualtrough, H. Worthington, D. B. Drucker. Antimicrobial activity of varying concentrations of Sodium hypochlorite on the endodontic microorganisms Actinomycesisraelii, A. naeslundii, Candida albicans and Enterococcus faecalis. International Endodontic Journal 2004;37: 438-446.

73. H. K. Haapasalo, E. K. Siren, T M T Waltimo, D .Orstavik, M.P.P. Haapasalo. Inactivation of local root canal medicaments by dentin: an in vitro study. Int Endod J 2000;33:126-131.

74. V.B Berber, B.P.F.A Gomes. N. T. Sena, C.C.R. Ferraz, A.A. Zaia, F. J. Souza–Filho. Efficacy of various concentrations of NaOCl and instrumentation techniques in reducing Enterococcus faecalis within root canals and dentinal tubules. Int Endod J 2006, 39: 10-17.

75. Krishna J N, Raghu R, Bolla N, Muddanna K. An in vitro comparative evaluation of the antibacterial efficacy of

10%metronidazole gel, 2% chlorhexidine gel and combination of calcium hydroxide and 2% chlorhexidine gel against Enterococcus facecalis. Journal of orofacial sciences 2012;4(1):26-31

76. Rahimi s, Janani M. A review of antibacterial agents in endodontic treatment. Iranian Endodontic Journal 2014;9(3):161-8
77. Prasad SD, Goda PC, Reddy KS, Kumar CS, Hemadri M, Ranga Reddy DS. Evaluation of antimicrobial efficacy of neem and Aloe vera leaf extracts in comparison with 3% sodium hypochlorite and 2% chlorhexidine against E. faecalis and C. albicans. J NTR Univ Health Sci 2016;5:104-10.
78. M.Asan Ozusaglam and K. Karakoca. Antimicrobial and antioxidant activities of Momordica charantia from Turkey. African Journal of Biotechnology 2013;12(13):1548-58.
79. Jahromi M Z, Boroujeni P M, Moshreffar A and Zamiran S. Effect of chlorhexidine 2% on shear bond strength of different composite resins to dentin. Int. j. Curr. Aca. Rev 2015;3(4):367-72.
80. Eswar K, Venkateshbabu N, Rajeswari K, Kandaswamy D. Dentinal tubule disinfection with 2% chlorhexidine, garlic extract and calcium hydroxide against Enterococcus faecalis by using real time polymerase chain reaction: in vitro study, J Cons Dent 2013;16(3):194-8.

81. Javidi M, Afkhami F. Efficacy of a combined nano particulate/ calcium hydroxide root canal medication on elimination of Enterococcus faecalis. Aust Endod J 2014;40:61-5.

82. Spangberg L, Engstrom B, Langeland K. Biological effects of dental materials, toxicity and antimicrobial effect of endodontic antiseptics in vitro. Oral Surg Oral Med Oral Pathol. 1973;36(6):856-71.

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This ethical committee has undergone the research protocol submitted by **Dr. Y. Anusha** Post Graduate Student, Department of Conservative Dentistry and Endodontics under the title **"A COMPARATIVE EVALUATION OF ANTIMICROBIAL EFFICACY OF BITTER GUARD (MOMORDICA CHARANTTIA) & GARLIC (ALLIUM SATIVUM) AS ENDODONTIC IRRIGANTS-AN IN VITRO STUDY** Reference No: 2015- MD-BrIV-SAT-08/APDCH under the guidance of **DR. S. Thillainayagam MDS.,** for consideration of approval to proceed with the study.

This committee has discussed about the material being involved with the study, the qualification of the investigator, the present norms and recommendation from the Clinical Research scientific body and comes to a conclusion that this research protocol fulfils the specific requirements and the committee authorizes the proposal.

Date: **Member secretary**